SYMPOSIUM: EVOLUTION OF COLEOID CEPHALOPODS

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Biochemical and molecular approach to cephalopod phylogeny

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Abstract: Cephalopod taxonomy is still uncertain, and little is known of the phylogeny of Recent taxa. Biochemical and molecular characters are complementary to morphology, and allow an additional insight into the phylogenetic relationships among cephalopods. Eye lens protein electrophoresis and immunological approaches yield data in agreement with traditional taxonomic grouping, but are less suitable for establishing phylogenetic relationships. Molecular tools, *e. g.* the 3' end of the 16S rDNA gene, have failed to resolve the phylogeny at the suprafamilial level, but seem appropriate at lower levels. DNA sequence comparisons (% substitution) show that a direct relationship between taxonomic rank and nucleotide divergence cannot be established, as the nucleotide divergence level differs from one taxa to the other.

Electrophoretic and immunological analyses of eye lens proteins as well as molecular results suggest that sepiolids should be separated from Sepioidea.

Phylogenetic analyses allow hypotheses of biological evolution based on various criteria to be tested. Morphological classification of taxa has, historically, been the first approach considered, and the analyses of anatomical structures are still of prime importance in the construction of hypotheses on the evolutionary history of organisms. But more recently, the development of biochemical and molecular techniques, that allow an insight into genetic structure, have opened new perspectives in taxonomy and phylogeny. Paleontology provides, however, the only direct means of calibrating evolutionary events. Both paleontology and embryology allow recognition of homologous characters derived from a common ancestor, the ancestors being deduced from the characters of terminal groups.

As far as cephalopods are concerned, the taxonomic status of many groups is still uncertain, and little is known of the phylogeny of Recent taxa. The main reason for this is that although the fossil record contains a wealth of ectocochleate forms (all extinct except *Nautilus*), this is not the case for Recent, mainly soft-bodied animals with a reduced or absent internal shell. So far only a few authors have considered the phylogenetic systematics of the group Cephalopoda. Fioroni (1981) was one of the first to use the Hennig (1966) approach to systematics. Berthold and Engeser (1987) established adelphotaxonomic relationships by the identification of synapomorphies in fossil and Recent taxa and proposed a phylogenetic classification of 35 subordinate taxa. The cladograms presented in these papers are not totally in agreement, drawing attention to the necessity of using additional criteria, such as biochemical and molecular, for taxonomic and phylogenetic reconstruction of cephalopod systematics.

All approaches, morphological, biochemical, and molecular, are complementary but each has advantages and disadvantages. Molecular analyses provide new tools to test the hypotheses based on morphology, and help to reformulate them in some cases. Morphological characters are more accessible, easier and less costly to analyze, allow comparison of extant and fossil forms, but can be more subjective. Biochemical and molecular characters are more objective, are potentially very abundant, but are not always easy to analyze. For instance, the fact that the nucleotides at each position can exist in only four states could be an important source of homoplasy. It is also well known that different portions of the same gene have not all the same probability of variation, and the rate of evolution can be different for the same gene in different taxa.

This paper is aimed to compare the results obtained using biochemical (electrophoretic and immunological) and molecular techniques for phylogenetic reconstruction of cephalopods using a number of species, comprising octopods and decapods.

MATERIALS AND METHODS

Fresh cephalopod tissue samples were obtained from various sources (Table 1). They were either stored at -20°C prior to electrophoretic and immunological analyses, or alcohol preserved for nucleotide sequencing.

Table 1. List of cephalopod species analyzed by eye lens protein electrophoresis and mtDNA sequencing. Their geographical origin is indicated as well as the source of the data: 1, Bonnaud *et al,* 1994; 2, Tranvouez and Boucher-Rodoni, 1990; X, present paper; (-), no data.

Origin	Species	Eye lens	mtDNA
East Atlantic (Biscay)	*Sepia officinalis* Linné, 1758	X	1
Mediterranean (Banyuls)	*S. orbignyana* Férussac, 1826	2	1
SW Pacific (New Caledonia)	*S. latimanus* Quoy and Gaimard, 1832	X	1
English Channel (Roscoff)	*Loligo vulgaris* Lamarck, 1798	2	1
SW Pacific (New Caledonia)	*Sepioteuthis lessoniana* Lesson, 1830	X	1
English Channel (Roscoff)	*Sepiola atlantica* Orbigny, 1840	X	1
Mediterranean (Banyuls)	*Rossia macrosoma* (Delle Chiaje, 1829)	X	1
Mediterranean (Banyuls)	*Todaropsis* sp.	X	(–)
Pacific Ocean (Hawaii)	*Todarodes* sp.	(–)	1
Mediterranean (Banyuls)	*Octopus vulgaris* Cuvier, 1797	2	(–)
Mediterranean (Banyuls)	*Eledone cirrhosa* (Lamarck, 1798)	2	1
SW Pacific (New Caledonia)	*O. cyanea* Gray, 1849	X	1
SW Pacific (New Caledonia)	*O.* sp.	X	(–)
East Atlantic (Mauritania)	*Graneledone verrucosa* (Verrill, 1881)	X	(–)
East Atlantic (Mauritania)	*Opistoteuthis agassizii* (Verrill, 1883)	X	(–)

For biochemical approaches (electrophoresis and immunology), the protein chosen should be stable enough that individual physiological changes do not influence the observed differences between species, but it should be variable enough to reflect taxonomic and phylogenetic differences. Eye lens proteins and hemocyanin were tested here.

Eye lens proteins were extracted according to the protocols described in Tranvouez and Boucher-Rodoni (1990), and analyzed on precast polyacrylamide gels (ExcelGel SDS Gradient 8-18%, Pharmacia). A band presence/absence matrix was computed and processed by the NTSYS-pc program (Rohlf, 1990), using Sahn clustering (Sneath and Sokal, 1973) with UPGMA and Neighbor-Joining (NJ) methods (Saitou and Nei, 1987), to produce phenograms. PAUP 3.1 (Swofford, 1985) was used to estimate phylogenetic trees.

Protein antigenic properties are supposed to allow the estimation of immunological distance between taxa (Tsusumi *et al.*, 1989). The ELISA immunological technique, an enzyme-linked immunosorbent assay, was adapted to cephalopod eye lens protein (Boucher-Rodoni *et al.*, 1995). Four eye lens antisera were available (*Sepia officinalis, S. orbignyana, Loligo vulgaris, Octopus vulgaris*). A homologous standard inhibition curve was determined with 2500-fold diluted serum, and the affinity of heterologous samples was then tested.

The antigenic properties of hemocyanin, the large respiratory protein found in the blood of all cephalopods, was also used here to estimate taxonomic relationships. The immunological distance of various cephalopod species was estimated by heterologous reaction against commercial keyhole limpet hemocyanin (KLH) and compared to preliminary results obtained with homologous antiserum.

For molecular analyses, attention was first focused on mitochondrial DNA (mtDNA) because of its diversity, and because data on various groups, including non-molluscan invertebrates, are well known. mtDNA is a small circular DNA molecule present in many copies in the mitochondria, it is maternally inherited, and there is no recombination. The protein genes and the r-RNA genes have both a mosaic structure of conserved and variable regions, which should allow phylogenetic relationships at various hierarchical levels to be analyzed. Nucleotide sequence data from the 3' end of the 16S rDNA gene have already been used to analyze phylogenetic relationships among decapod cephalopods (Bonnaud *et al.*, 1994). To determine whether the different taxonomic hierarchical levels can be related to a given nucleotide percentage of divergence, some sequences from two populations of the same species, from different species of the same genus, and from different genera, were compared and analyzed in terms of molecular divergence (% substitution).

RESULTS

The results of eye lens protein electrophoresis of 14 cephalopod species analyzed by UPGMA and Neighbor-Joining methods show that with both methods the taxonomic grouping is respected (Fig. 1). Octopods are always grouped together, but are not distinctly separated from decapods. Incirrate octopods are a sister group of cirrate octopods, but at lower taxonomic levels the genus *Octopus* is not homogeneous. As far as decapods are concerned, the relationships among myopsids, oegopsids, and sepioids are not clearly defined by either approach. The sepiolids occupy a particular position in the NJ analysis, branching as a sister group of all other coleoids. The strict consensus tree derived from the phylogenetic analysis (PAUP 3.1) is less

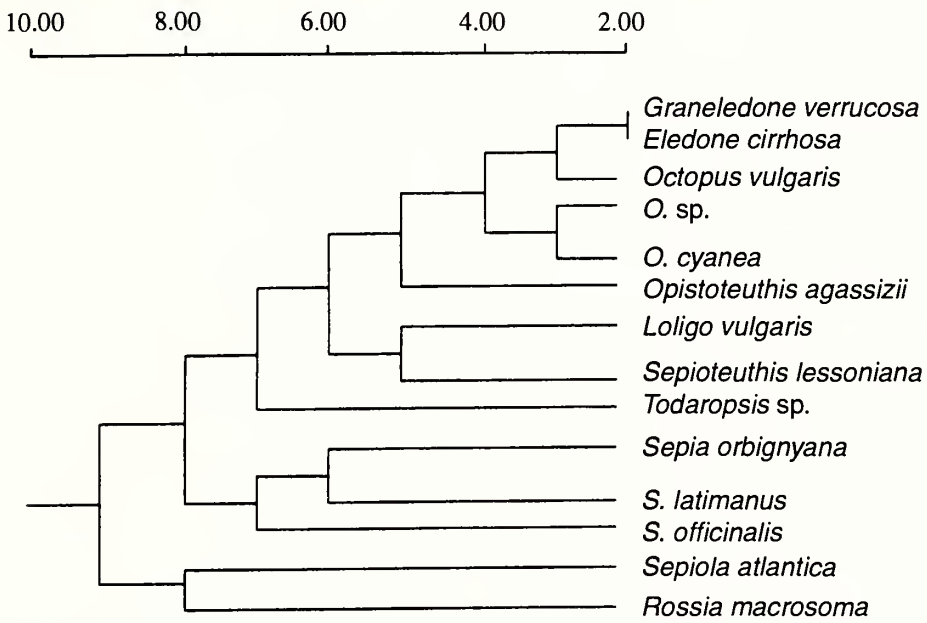

Fig. 1. Phylogenetic tree obtained by the Neighbor-Joining method on SDS-Page electrophoretic analysis of eye lens proteins.

resolved for octopods (Fig. 2).

When using protein eye lens immunological properties to estimate immunological distances for phylogenetic analyses, one of the main problems encountered was that in some cases the distances were not symmetrical. Table 2 shows that when comparing the heterologous reaction between two species using each species extract in turn as antiserum and antigen, the immunological distance is not necessarily the same.

The hemocyanin is currently used as an immunogenic agent. In cephalopods it is composed of seven functional units in octopods and *Nautilus*, and eight functional units in decapods (Van Holde *et al.*, 1992). Fig. 3 shows the immunoreactivity of the hemocyanin of various cephalopod species against KLH antiserum, estimated by the ELISA technique. *Nautilus* reactivity was the closest to KLH homologous reaction, most of the other species being grouped together at a rather distant position, except *Sepia* which displayed a reactivity stronger than all other coleoids. Preliminary assays with homologous hemocyanin antiserum indicate that the distances are readable at high hierarchical levels (*i. e.* distant taxa), but do not discriminate species. The distance between *Sepia* and *Nautilus* was the smallest, but the difference was not as important as with KLH. And again, as with eye lens proteins, the results were not symmetrical.

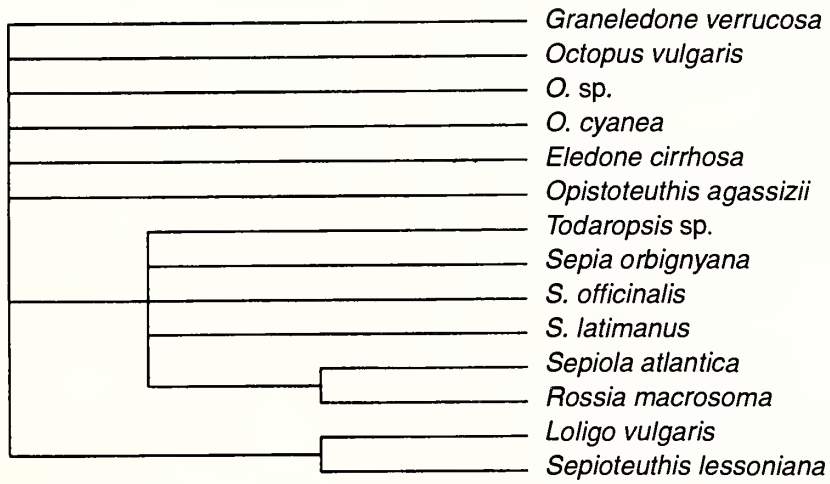

Fig. 2. Strict consensus tree (unrooted) of 100 trees obtained by heuristic search (PAUP 3.1; MULPARS option).

Table 2. Results of immunological heterologous reaction (ELISA technique) between species pairs using each species extract in turn as antiserum and antigen. Values represent immunological distances estimated by optical density differences.

ANTISERUM		ANTIGEN
Sepia officinalis	4 / 4	*S. orbignyana*
S. officinalis	10 / 15	*Loligo vulgaris*
L. vulgaris	17 / 13	*S. orbignyana*
Octopus vulgaris	16 / 25	*S. officinalis*
O. vulgaris	15 / 17	*S. orbignyana*
O. vulgaris	24 / 25	*L. vulgaris*

To analyze the relationships of cephalopods at a perispecific level (population, species), many authors have studied enzymatic polymorphism which remains a valuable tool for genetic population analyses (Levy *et al.*, 1988; Carvalho *et al.*, 1992; Brierley *et al.*, 1993, 1995).

If mtDNA is considered, preliminary results on six different populations of *Sepia officinalis* indicate that neither 16S, nor cytochrome oxidase CoII or CoIII, displays adequate variability for taxonomic purposes, whereas enzymatic polymorphism does (Bonnaud, unpub. data). Accordingly, when comparing the 3' end of 16S rDNA gene sequences, in terms of % substitution, the difference between two distant populations of *S. officinalis* (Mediterranean and English Channel) is not significant (Table 3). Therefore, this gene portion is not appropriate to provide evidence of differences among *S. officinalis* populations.

At higher taxonomic levels, the status of many cephalopod groups is still uncertain and needs to be reconsidered with the help of phylogenetic reconstruction. Phylogenetic hypotheses to estimate divergence time are usually given through paleontology (Doyle *et al.*, 1994). True cuttlebones are only known from the early Tertiary; Teuthoidea and Sepioidea are thus supposed to have diverged in the Cenozoic.

The use of mtDNA 16S for phylogenetic reconstruction was shown to be appropriate to test Tertiary divergences in insects and vertebrates (Simon *et al.*, 1990; Hillis and Dixon, 1991), but such is not the case for cephalopods (Bonnaud *et al.*, 1994). The phylogeny of cephalopods is unresolved at the suprafamilial level because of excessive nucleotide divergence (saturation), perhaps due to earlier emergence than Cenozoic, or to unequal evolutionary rates among taxa.

To compare results derived from protein elec-

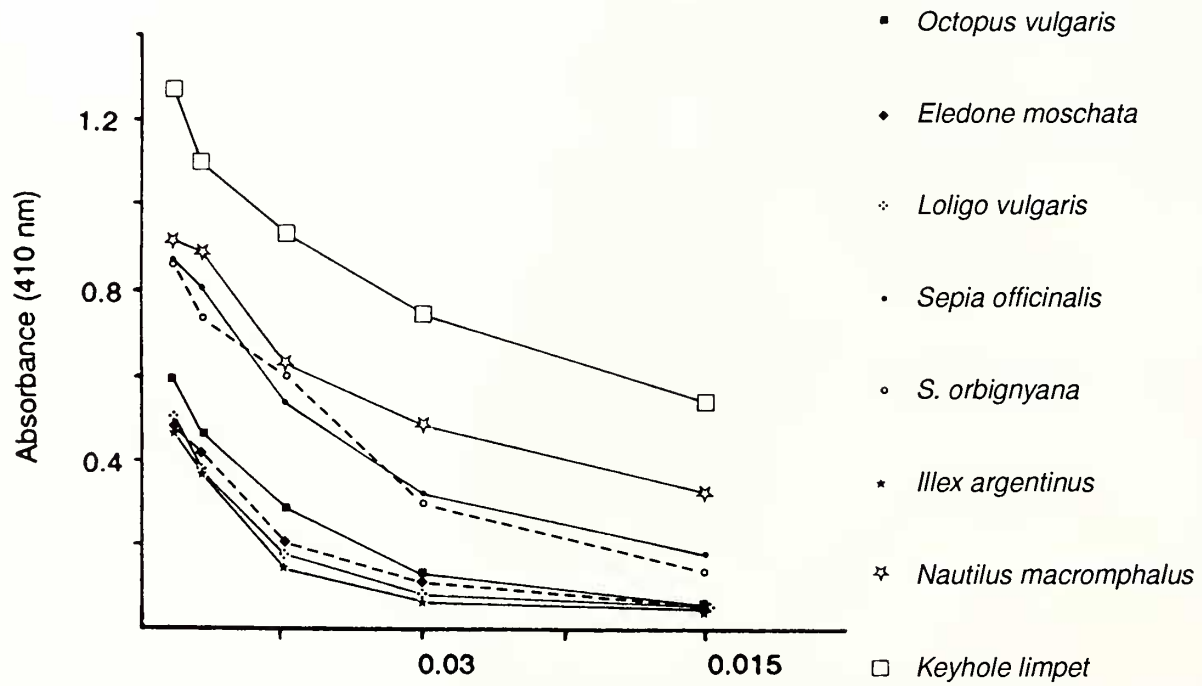

Fig. 3. Protein (µg/ml) Immunoreactivity of hemocyanin of various cephalopod species against keyhole limpet hemocyanin antiserum, tested by the ELISA technique.

Table 3. Nucleotide divergence of 3' end of 16S rDNA gene sequences at various taxonomic levels: (a) populations, (b) intrageneric, (c) intergeneric.

	Species	Divergence (% substitution + gaps)
(a)	*Sepia officinalis* (Roscoff) - *S. officinalis* (Banyuls)	*ca.* 1
(b)	*S. officinalis* - *S. orbignyana*	13.8
	S. orbignyana - *S. elegans*	8.1
	S. elliptica - *S. pharaonis*	10.3
	S. pharaonis - *S. smithi*	8.8
	Loligo forbesi - *L. vulgaris*	9.4
	Nautilus macromphalus - *N. pompilius*	5.5
(c)	*Sepietta* sp. - *Sepiola atlantica*	5.3
	Sepietta sp. - *Rossia macrosoma*	13.0
	Sepiola atlantica - *Rossia macrosoma*	12 1
	Sepietta sp. - *Sepia officinalis*	17.9
	Sepietta sp. - Loligo vulgaris	18.4*

*Overestimated value, because of an insertion of *ca.* 20 bases in *Loligo*.

trophoresis and sequence data from the 3' end of 16S rDNA, the sequences corresponding to some of the taxa appearing in Fig. 1 were aligned and analyzed by Neighbor-Joining and PAUP methods. Both approaches show that nucleotide analysis clearly separates octopods and decapods, but again the monophyly of the order Sepioidea, including sepiids and sepiolids is not supported, the sepiolids being excluded from the order (Fig. 4). In terms of % substitution, sequence comparison shows that sepiolids are as distant from *Sepia* (17.9%) as from *Loligo* (18.4%) (Table 3). Intrageneric divergence ranges from 8 to 14% for sepiids (the highest value concerning *S. officinalis* and *S. orbignyana*), but a direct relationship between taxonomic rank and nucleotide divergence cannot be established, as the nucleotide divergence level in other taxa could be in another range. Between sepiolid genera, for instance (Table 3), the intergeneric divergence is 5.3% between *Sepiola* and *Sepietta*, two morphologically closely related species, but can be as high as 13.0% between *Sepietta* and *Rossia*. One surprising result concerns the sequence comparison of two morphologically distinct species of *Nautilus* which display a low nucleotide divergence value (5.5%).

DISCUSSION AND CONCLUSION

The reliability of eye lens proteins as a taxonomic tool was shown by some authors (Smith, 1969; Swanborn, 1971; Brahma and Lancieri, 1979; Tranvouez and Boucher-Rodoni, 1990). It is confirmed here that eye lens protein electrophoresis analysis serves to group closely related taxa together, but its use for inferring phylogenetic relationships leads to more questionable results. The use of immunological properties of eye lens proteins and of hemocyanin to analyze taxonomic relationships is rather disappointing, mainly because the distances measured by the ELISA technique between taxa are not symetrical, *i. e.* the distance between *Sepia* and *Nautilus* is not the same as the distance between *Nautilus* and *Sepia*. The immunological distance indicates the degree of similarity, but it is not a character that is easy to precisely quantify for phenetic or phylogenetic analyses.

The development of molecular biology has raised great hope and excitement about phylogenetic reconstructions. We now have direct access to the genetic material. However the enormity of the available genetic information is itself a problem: where is the most appropriate place to investigate to answer our questions? Rates of evolution differ from one gene to the other, and the main problem is to find a genetic marker appropriate for the hierarchical level we are interested in. If a gene has evolved too rapidly, it will be saturated with substitutions and provide a non-significant result. If it is too stable, the variability will not be informative enough. Our knowledge of genetic structures comes primarily from results obtained with vertebrates or *Drosophila*, but their levels of nucleotide divergence are not necessarily adequate for analysis of other phylogenetic relationships. This cannot be known *a priori*, and a series of prerequisites are necessary before starting a molecular analysis. (1) Choice of the gene: its variability must be adequate to the hierarchical level being considered. (2) Choice of the species (number and "quality"): this is important because some cephalopod genera include over 100 species, whereas others are monotypic. (3) Length of the sequence: apparently, bootstrap values increase with length of sequence, but the reliability depends rather on the number of taxa (Lecointre *et al.*, 1993). So, as with morphology, we will have to increase, as much as possible, the number of species for each taxon. This is obviously impossible when some genera or even families are monospecific. (4) Choice of an outgroup: it should comprise more than one species of a monophyletic group, close enough to the investigated taxon to preclude saturation, but sufficiently different to prevent inclusion in the ingroup.

As far as cephalopods are concerned, nucleotide sequence analysis provides a more reliable picture than electrophoresis and immunology which are interesting taxonomic tools, but are not satisfactory for phylogenetic analyses. However, a direct relationship between taxonomic rank and % nucleotide divergence cannot be established, as the nucleotide divergence level is different in different taxa.

The congruence between morphological and molecular analyses, two independent sets of data, is very important in construction of evolutionary patterns. Morphological-molecular comparisons however are still rare, and

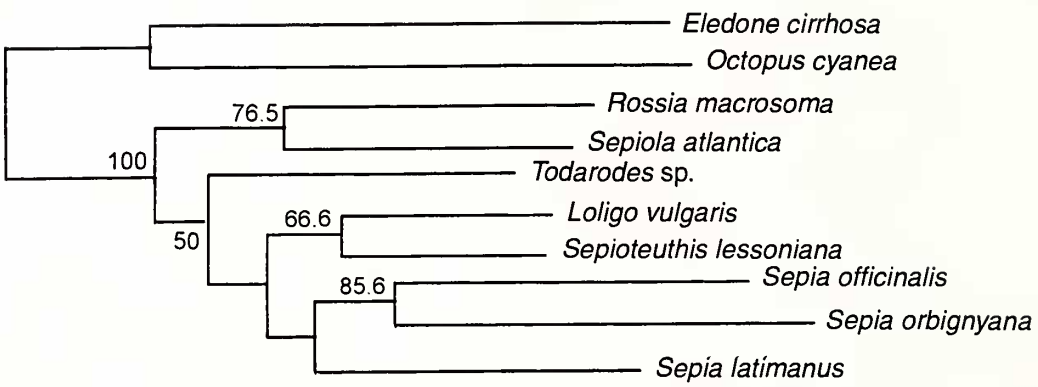

Fig. 4. Phylogeny inferred by Neighbor-Joining distance analysis from mtDNA 16S sequences (Bonnaud *et al*, 1994). Bootstrap values ≤ 50 are indicated.

not often congruent. In cephalopods, one solid and congruent result concerns sepiolids. Their taxonomic and phylogenetic position has been a matter of much discussion, being classified either as a family of the Sepioidea (Naef, 1912; Voss, 1977; Mangold and Portman, 1989) or as a family of the Myopsida together with the Sepiidae (Berthold and Engeser, 1987). Fioroni (1981, based on embryology) and Clarke (1988, based on morphology) proposed to raise the sepiolids to ordinal rank. The present electrophoretic, immunological, and molecular results confirm that sepiolids can be separated from the Sepioidea.

The analysis of phylogenetic relationships among coleoids must take into account a number of difficulties, whatever the criteria used, morphological, biochemical, or molecular: (1) poor fossil remains of recent taxa; (2) no outgroup: we choose octopods as an outgroup for decapods, and *vice versa* because, even if the distance is very important, we have no real alternative; (3) many monospecific genera; (4) traditional classification not well stabilized; (5) little information on rate and modalities of molecular evolution: the evolutionary rate of the different genes is inferred mainly from vertebrate results - vertebrates represent only one episode of the saga of life and it is not always possible to transpose to invertebrates genetic postulates based on vertebrate results.

To better understand higher hierarchical levels of phylogeny, it appears necessary in many cases to consider more than one gene, preferably by associating mitochondrial and nuclear information.

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Relationship of some coleoid cephalopods established by 3' end of the 16S rDNA and cytochrome oxidase III gene sequence comparison

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Abstract: Phylogenetic relationships for extant cephalopods have been based, so far, mainly on morphology and paleontology. Nucleotide sequence data are still rare. Sequence analyses from the 3' end of the 16S rDNA gene of cephalopods have shown that this portion of gene can provide valuable information on taxonomic relationships at the infrafamilial level. Another mitochondrial gene, cytochrome oxidase III, is investigated to analyze higher (*i. e.* ordinal) taxonomic levels. The results obtained by the two gene portions are compared, but the low number of species does not allow a definitive answer on interfamilial relationships. The low divergence between nucleotide sequences of two populations of *Loligo vulgaris* Lamarck, 1798, and of *L. reynaudii* Orbigny, 1845, suggests that the latter is not a clearly distinct species. The grouping of the three families of Sepioidea (Sepiidae, Spirulidae, and Sepiolidae) is not supported. Idiosepiidae groups with the oegopsid squid *Enoploteuthis* irregardless of the analysis (parsimony or distance).

The Decapoda are composed of two orders, Teuthoidea and Sepioidea. Relationships within these orders are not stabilized based on morphological characters. It is now possible with molecular characters to obtain a new type of information which can help to resolve some phylogenetic relationships. The 3' end of the mitochondrial- *l-r-*RNA (16S) was already investigated but with this portion of the molecule, extensive nucleotide variability leads to an unresolved phylogeny between the orders (Bonnaud *et al.*, 1994). Another mitochondrial gene, coding for cytochrome oxidase III (COIII), was chosen here to analyze the relationships between some species of decapods. The impact of mutations on protein function is very important, and accordingly, the structure of some genes coding for proteins should be less variable at the nucleotide level: this is the case for the genes coding for cytochrome oxidase subunits, COI, COII, and COIII. COIII gene analyses were thus thought to be suitable for solving phylogenetic relationships at hierarchical taxonomic levels higher than those resolved by the 16S gene. For this preliminary study, one species in each family or suborder was analyzed and the results obtained with the two gene portions compared.

MATERIAL AND METHODS

Details on the taxonomic position and origin of the eight species studied are presented in Table 1. DNA was extracted from frozen or alcohol-preserved tissues according to the protocol described in Bonnaud *et al.* (1994). A portion of 16S and a portion of COIII were amplified with universal primers: 984 and 986 for 16S and COIIIa and COIIIb according to the classification of Simon *et al.* (1991). These portions were cloned in pBS+ (Stratagène) and sequenced (ca. 500 pb each) with the dideoxy chain termination (Sanger *et al.*, 1977). The alignments were performed by eye, with the aid of secondary structure for 16S and of the reading coding frame for COIII. Phylogenetic trees were calculated by distance (Neighbor-Joining) method using the MUST package (Philippe, 1992) and parsimony method using PAUP 3.1 (Swofford, 1990). These two methods gave similar results and only the trees obtained with the distance method are described here. The robustness of internal branching was tested by bootstrapping.

Transversions (changes of pyrimidine to purine or *vice versa*) are known to be less abundant than transitions (changes of purine to purine or pyrimidine to pyrimidine) in some vertebrate taxa. When sequences are highly variable, transitions can introduce noise in the analyses. As a consequence, the use of transversions should lower the incidence of homoplasy between distant taxa. Analyses were performed using both all the substitutions and only the transversions. Attributing weight to the transversions instead of removing the transitions did not further change the results obtained.

Table 1. Geographical origin and systematic position of species studied.

SPECIES (ORIGIN)	SYSTEMATIC POSITION	
Sepia officinalis Linné, 1758 (Banyuls)	Sepiidae	SEPIOIDEA
Sepietta sp. (Banyuls)	Sepiolidae	
Spirula spirula (Linné, 1758) (New Caledonia)	Spirulidae	
Idiosepius pygmaeus Steenstrup, 1881 (Australia)	Idiosepiidae	
Enoploteuthis reticulata Rancurel, 1970 (Hawaii)	Enoploteuthidae (Oegopsid squid)	TEUTHOIDEA
Loligo vulgaris Lamarck, 1798 (Roscoff)	Loliginidae (Myopsid squids)	
L. vulgaris (Banyuls)		
L. reynaudii Orbigny, 1845 (South Africa)		
Octopus cyanea Gray, 1849 (New Caledonia)	Octopodidae	

RESULTS

Analysis of partial 16S gene

Analyses were performed with 131 informative sites out of 231 variable sites. In agreement with the results obtained previously (Bonnaud *et al.*, 1994) the tree issued from the analysis of *l-r*-RNA gene is unresolved (Fig. 1). No strong relationship can be established among taxa, except for the Loliginidae. This is correlated with the sequence identities: the sequences of the two *Loligo vulgaris* populations are identical and that of *L. reynaudii* differs by only 1.1%, a percentage close to the error percentage generally accepted after amplification, cloning, or sequencing. The general topology of the tree appears coherent with the classification issued from morphological data (*i. e. Idiosepius, Sepia,* and *Sepietta* grouped together) but none of the groupings is strongly supported by a high bootstrap value. *Idiosepius* is not more closely related to the Sepioidea than to the Teuthoidea. It must be stressed that a complementary analysis with all available species did not provide a clearer answer, the substitutions between families being saturated (Bonnaud *et al.*, 1994): *Idiosepius'* position cannot be determined.

Analysis of partial COIII gene

Analyses were performed with 166 informative sites out of 258 variable sites of a portion of cytochrome oxidase III gene. The trees obtained with nucleotide sequence analysis (Figs. 2-3) likewise show a solid grouping of the three loliginids. The sequences of the two populations of *Loligo vulgaris* differ significantly with this gene (4.9% of nucleotide divergence), and the divergence between *L. vulgaris* "Roscoff" and *L. reynaudii* was 6.3%. Their grouping was always supported by a very high bootstrap value (100).

Another well-supported group is composed of the oegopsid squid *Enoploteuthis reticulata* and of *Idiosepius pygmaeus*, the representative of Idiosepiidae, one of the sepioid families. This same grouping of *Idiosepius* and *Enoploteuthis* was obtained when taking all the substitutions into account or only the transversions. Grouping of the three other families of Sepioidea (Sepiidae, Spirulidae, and Sepiolidae) is not supported. This was confirmed by the separation of these three species in the PAUP analyses including all the substitutions or with weighted transversions (data not shown). When taking into account only the transversions, *Spirula* became linked with *Loligo* with a bootstrap value of 57.9 with Neighbor-Joining as well as with PAUP analyses, and the group excluding *Sepietta* and *Sepia* was well supported.

DISCUSSION AND CONCLUSION

It is clear that the two *Loligo* species are difficult to distinguish in terms of nucleotide variability with the

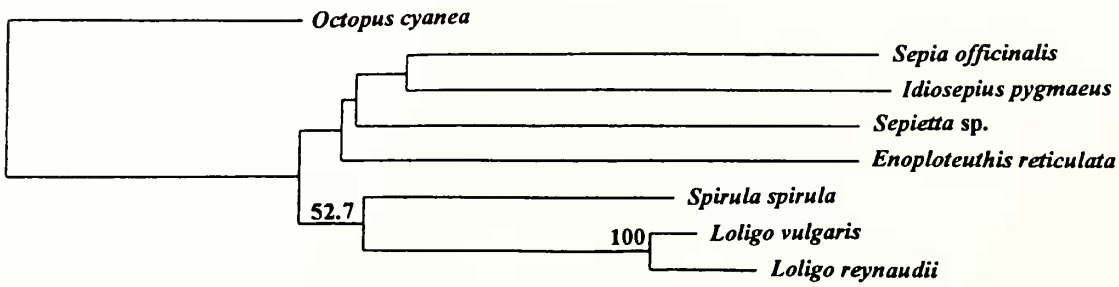
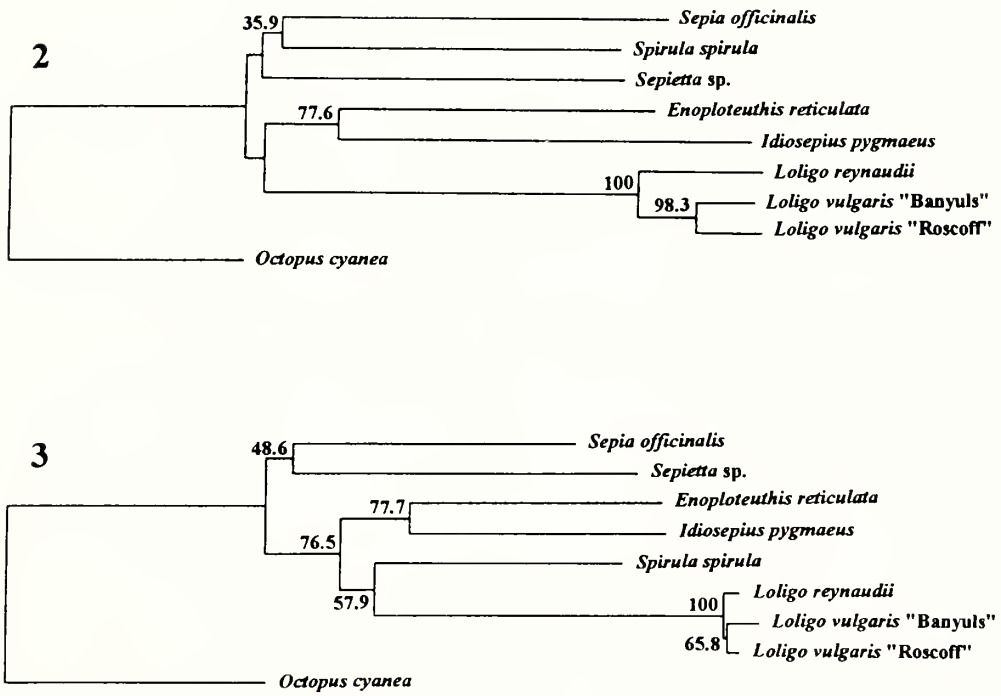


Fig. 1. Phylogenetic tree from the analysis of the 3' end of *l-r*-RNA using all substitutions (Neighbor-Joining method).



Figs. 2-3. Phylogenetic trees from analyses of partial COIII gene using (2) all the substitutions, (3) only the transversions (Neighbor-Joining method).

portions of the two mitochondrial genes. The observed divergence suggests differences due to geographical separation rather than a well-established speciation event. A study involving numerous specimens from along the French and African coasts would certainly provide an answer to this hypothesis of clinal variation.

The analysis of transversions only must be viewed with precaution, especially when the group excluding *Sepietta* and *Sepia* is considered. It is clear that the number of species was limited. In PAUP analyses of the COIII portion, *Sepietta* was linked with *Idiosepius* and *Enoploteuthis* whatever substitutions were considered. The opposing results from the two methods for the grouping of *Sepietta* reveal that this relation is uncertain and needs confirmation. The same is true for *Spirula* grouped with *Loligo*. The bootstrap values of 52.7 (with 16S) and 57.9 (with COIII) are low. If these values are really significant, they could be modified by increasing the species sampling, when possible.

On the contrary, *Idiosepius pygmaeus* is always grouped with the oegopsid irregardless of the analysis (parsimony or distance). This was unexpected because Idiosepiidae was placed by Naef (1916) with the Sepiidae and Sepiolidae as members of the order Sepioidea. For most authors, *Idiosepius* is more closely related to the Sepiolidae and Sepiadariidae than to the Sepiidae or Spirulidae, and its phylogenetic position has been questioned so far only with regard to the first two families (Fig. 4). The taxonomic rank of the idiosepiids has rarely been changed: it was always considered as a family of the order Sepioidea except by Guerra (1992) who raised Idiosepiidae to ordinal rank. The idiosepiids are isolated by characters like a dorsal adhesive organ in adults, the retardation of the tentacle development in juveniles, and the statocyst structure. They were often described without shell or gladius. The presence of a gladius was certainly difficult to detect because of the very small size of the members of this genus (20 mm maximum mantle length). Hylleberg and Nateewathana (1991a, b) found a thin gladius in the specimens examined and suggested that *I. pygmaeus* might be more closely related to Teuthoidea than to Sepioidea. Steenstrup (1881) created the genus *Idiosepius*; in his original description he mentioned that some specimens of small squids were described by early authors (*e. g.* Lamarck, Orbigny, Férussac, Blainville, Péron, Lesueur) under various names: *Cranchia minima* Férussac, 1835, *Loligo minima* Orbigny, 1848, *Loligopsis peronii* Lamarck, 1822, *Loligo parvula* Péron in Blainville, 1823, *Sepiola minima* Lesueur, 1821. In the opinion of Steenstrup, these decapods might be idiosepiids. The reasons which lead these early authors to attribute squid characteristics to *Idiosepius* could be an indication of the peculiar position of this genus within Decapoda. It is difficult to find morphological criteria which justify linking *Idiosepius* with sepiolids or sepiids and the few existing morphological studies, like those of Hylleberg and Nateewathana (1991a, b), do not analyze the taxonomic status of *Idiosepius*.

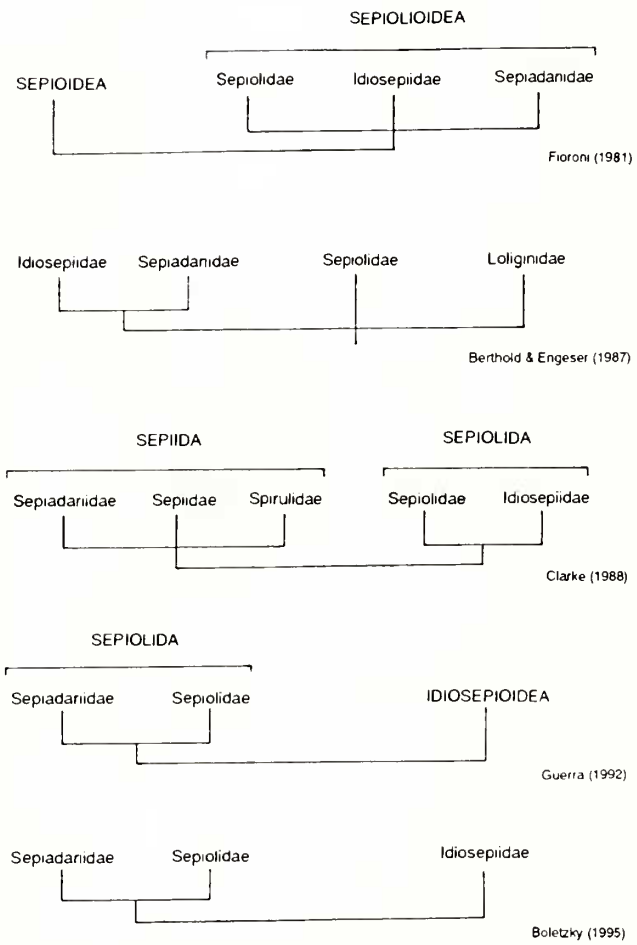


Fig. 4. Relationships of idiosepiids with other taxa according to various authors. Ordinal rank indicated by capital letters.

Analysis of additional species, and especially of other oegopsid families, might help to confirm the unexpected position of *I. pygmaeus*, and eventually to relate it more closely to an oegopsid family (Bonnaud *et al.*, in press).

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Analysis of morphology to determine primary sister-taxon relationships within coleoid cephalopods

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Abstract: Although most families of living coleoid cephalopods are well defined, phylogenetic relationships among them are controversial. A necessary first step toward analyzing the phylogeny of decapod families is the determination of proper outgroups to polarize characters. The cladistic position of the Vampyromorpha is of particular interest. Toward this goal, we have examined 50 morphological characters in 24 species from 17 families. The material examined included representatives from the oegopsids, myopsids, sepioids and sepiolids, cirrate and incirrate octopods, and *Vampyroteuthis*. At this level, the characters were polarized either by comparison with *Nautilus*, or, for a few, by ontogenetic sequence or the fossil record. We found that of these 50 characters, 25 could not be used with confidence because of problems primarily involving character independence, apomorphic "loss," or assessment of homology/homoplasy. The states of three characters could be assumed to be ordered. Only ten characters were unambiguously informative as defining synapomorphies at the ordinal level. The resulting consensus of most-parsimonious trees is: (((oegopsid + myopsid + sepioid + sepiolid)(((cirrate)(incirrate)) vampire))(nautilus)).

Although most families of living coleoid cephalopods are well defined, phylogenetic relationships among them are controversial. Our understanding of cephalopod phylogeny is based mostly on the work of Naef (1921-1923; 1928). Advances in cephalopod systematics since the 1920s have mainly described new orders, families, genera, and species. The unraveling of the evolutionary pathways of this group has remained nearly stagnant. The phylogenetic classification presented by Voss (1977), summarized from much earlier authors, has been widely followed by most workers studying extant cephalopods (*e. g.* Roper *et al.*, 1984; Nesis, 1987; Mangold and Portmann, 1989; and the many researchers who have used their works). Many paleontologists, however, have not been comfortable with this classification. Relationships among the decapods have also been questioned (*e. g.* J. Z. Young, 1977; Fioroni, 1981; Boletzky, 1993a). Recently, Berthold and Engeser (1987) performed a phylogenetic analysis of the coleoid cephalopods and the resulting classification was quite different from that given by Voss. Perhaps the most important difference is that the myopsid squids (Loliginidae and Pickfordiateuthidae) were found to be more closely related to the cuttlefishes than they are to the other squids. Other recent attempts based on limited sets of characters (*e. g.* Clarke and Maddock, 1988; Clarke, 1988; Khromov, 1990) have not produced a credible genealogy of coleoid cephalopods.

The fossil record of coleoid cephalopods is generally meager with the exception of the hard structures of belemnites. Indeed, Donovan (1977: 45) concluded that "The phylogeny of living coleoids has to be compiled from hopelessly inadequate paleontological evidence. It is not surprising that attempts to work it out have been made at long intervals and have been more or less unconvincing." Much undescribed and unexamined fossil "teuthoid" material exists in museums (Donovan, 1977). Perhaps sufficient material exists that a convincing case for relationships could be made if interpreted within the general framework of a genealogy based on the analysis of Recent cephalopods.

Our virtual lack of progress in understanding cephalopod evolution is due to the fact that: (1) no broad-based morphological study has been attempted since Naef's work, although several dissertations on the comparative morphology of particular structures have produced valuable information (*e. g.* Toll, 1982, teuthoid gladius; Brakoniecki, 1986, loliginid hectocotylus; Hess, 1987, spermatophores; S. Candela, University of Miami, in preparation, beaks); (2) only a single molecular study of higher-level systematics has been published (Bonnaud *et al.*, 1994), and its results demonstrated the difficulty in selecting proper genes for analysis; and (3) studies using cladistic techniques have been few and these have dealt with genera within a family (*e. g.* Voss and Voss, 1983) or between just a few families

(R. E. Young and Harman, in press). Cladistic techniques on a broad basis have not been properly used by either neontologists or paleontologists.

As a first-step toward analyzing cephalopod phylogeny we have chosen a top-down approach in order to determine outgroups for polarizing characters at lower phylogenetic levels. Our goals in this paper are: (1) to determine if the major coleoid taxa, Cirrata, Incirrata and Decapoda, are monophyletic (the monophyly of the Vampyromorpha is already established by its monotypy); and (2) to determine the relationships among these four taxa. Achievement of these goals will determine the placement of the Vampyromorpha and whether or not the Octopoda is monophyletic. We have examined 50 morphological characters in 24 species from 17 families to determine these ordinal and subordinal relationships. The material examined included representatives from the oegopsids, myopsids, sepioids and sepiolids, cirrate and incirrate octopods, and *Vampyroteuthis*.

MATERIALS AND METHODS

As terminal taxa for this study, we selected 17 families which we felt were representative of the putative major groups of extant coleoids although we did not include families that have highly derived autapomorphies (*e. g.* the coelomic specializations of cranchiid squids). Most families, other than monotypic families, were represented by two species often in two genera (Table 1). These species were examined for each character from specimens in the USNM collections (National Museum of Natural History, Washington, D. C.). In addition, a large variety of individuals was examined to determine the structure of a character in order to refine the character definition.

We surveyed 50 characters for each of the terminal taxa. The characters and their states are presented below in the Results section. Almost all character states were assessed by direct examination of specimens. In a few cases, such as neuroanatomy of the brain, we accepted reliable observations from the literature. As discussed below, half of the characters were eliminated from final analyses because of questions about independence, problems with assessing homology versus homoplasy, or our inability to define and assess unambiguous character states.

Nautilus is the clear outgroup for the coleoids. However, because *Nautilus* is so far removed from the coleoids morphologically, many of the ingroup characters are not applicable to it. In order to incorporate information from paleontology and ontogeny for polarity determination, we designated the outgroup as *Nautilus*/ancestral-coleoid. Thus, when a character was not applicable to *Nautilus* but could be polarized by fossil or developmental observations,

Table 1. Species examined for all characters. ML = mantle depth.

FAMILY (HIGHER TAXA) Species	USNM Catalog No.	ML	Sex
BATHYTEUTHIDAE (OEGOPSIDA)			
Bathyteuthis abyssicola Hoyle, 1885	885673	63	female
	577804	40	male
	577804	53	female
BOLITAENIDAE (INCIRRATA)			
Japetella diaphana Hoyle, 1885	885674	41	juv.
	575636	80	female
J. heathi (Berry, 1911)	813756	40	juv.
ENOPLOTEUTHIDAE (OEGOPSIDA)			
Abralia trigonura Berry, 1913	730630	41	female
	730630	35	male
Enoploteuthis anapsis Roper, 1964	728753	62	male
	728753	81	male
	728754	72	female
	728754	40	female
GONATIDAE (OEGOPSIDA)			
Gonatus antarcticus Lönnberg, 1898	885675	139	female
LOLIGINIDAE (MYOPSIDA)			
Loligo pealei Lesueur, 1821	814246	160	male
Lolliguncula brevis (Blainville, 1823)	729175	104	female
Sepioteuthis sepioidea (Blainville, 1823)	814383	140	female
NAUTILIDAE (NAUTILOIDEA)			
Nautilus pompilius Linné, 1758	678868	70	female?
OCTOPODIDAE (INCIRRATA)			
Octopus vulgaris Cuvier, 1797	577100	48	female
OCYTHOIDAE (INCIRRATA)			
Ocythoe tuberculata Rafinesque, 1814	727831	62	female
OMMASTREPHIDAE (OEGOPSIDA)			
Illex illecebrosus (Lesueur, 1821)	885676	115	male
	885676	128	juv.
Ommastrephes bartramii (Lesueur, 1821)	814773	109	juv.
	814773	113	---
	814773	111	male?
ONYCHOTEUTHIDAE (OEGOPSIDA)			
Onychoteuthis banksii (Leach, 1817)	727524	93	female
OPISTHOTEUTHIDAE (CIRRATA)			
Opisthoteuthis agassizi Verrill, 1883	817405	27	female
O. californiana Berry, 1949	575640	31	female
SEPIIDAE (SEPIOIDEA)			
Sepia officinalis Linné, 1758	817479	84	male
SEPIOLIDAE (SEPIOIDEA)			
Rossia pacifica Berry, 1911	214611	33	female
Sepiola atlantica Orbigny, 1839-1842	575463	16	female
	575463	15	male
SPIRULIDAE (SEPIOIDEA)			
Spirual spirula (Linné, 1758)	814005	42	female
STAUROTEUTHIDAE (CIRRATA)			
Stauroteuthis syrtensis Verrill, 1879	817381	54	female?
	817383	50	not det.
THYSANOTEUTHIDAE (OEGOPSIDA)			
Thysanoteuthis rhombus Troschel, 1857	730192	147	male
VAMPYROTEUTHIDAE (VAMPYROMORPHA)			
Vampyroteuthis infernalis Chun, 1903	885677	55	female

these states were entered. When a character was not applicable to *Nautilus* and fossil/ontogenetic information was lacking, a "?" was entered in the data matrix.

Cladistic analyses were calculated using PAUP, version 3.1.1 (Swofford, 1993) and checked with Hennig86, version 1.5 (Farris, 1988) because the two programs treat data slightly differently in some circumstances (Platnick *et al.*, 1991). All characters were unweighted. In PAUP, a heuristic search was run utilizing the random stepwise addition sequence and the tree bisection-reconnection branch-swapping algorithm. To insure locating the shortest trees, 100 replicates were run, and a strict consensus was performed on all minimum-length trees. Hennig86 analyses used the "ie*" option to utilize all available tree space, and then a Nelsen consensus tree was calculated for the results. When a family was polymorphic for a character, this was entered as a separate state in Hennig86, as opposed to polymorphic coding for PAUP.

The consensus trees were analyzed with MacClade, version 3.0 (Maddison and Maddison, 1992) for impossible character polarization resulting from the use of "?" for unknown or inapplicable characters in the *Nautilus*/ancestral-coleoid taxon. No such cases were found. Information on character transformation was taken from analyses in MacClade. Data on the ingroup and outgroup were analyzed simultaneously and unrooted, then subsequently rooted between the ingroup and outgroup (Nixon and Carpenter, 1993).

RESULTS

CHARACTER DESCRIPTIONS

Character No. 1: Siphuncle. Character states: 0 - absent; 1- present.

Comments. The presence of a siphuncle is well known in *Sepia, Spirula, Nautilus,* and numerous fossil cephalopods and state 1 (present) is clearly the plesiomorphic condition in coleoids. Less well known is the possible remnant of the siphuncle in *Vampyroteuthis*. In *Vampyroteuthis* a long, very slender duct, continuous with the visceropericardial (VP) coelom, extends posteriorly from the coelom to an expanded but flattened sac that sits within the apex of the gladius (Fig. 1A). The posterior wall of the sac is complex histologically on its exterior surface but the function is unknown. The pigmented coelomic epithelial lining makes the thread-like duct visible. Pickford (1940) believed this duct to be a remnant of the siphuncle. The siphuncle of living cephalopods contains an extension of the VP coelom and the duct in *Vampyroteuthis* arises in the position where the siphuncle would be expected (*i. e.* body midline). Alternatively the duct could represent the first stage in the reduction of the coelom leading to the octopod condition. In octopods, narrow ducts, found in other locations, resulted from the reduction of the coelom (*i. e.* the "water canals") (see Character 20). In *Vampyroteuthis* the coelom proper terminates well in advance of the conus of the gladius, a condition not found in decapods. Understanding the structure and function of the "end organ" in *Vampyroteuthis* might help resolve the homology of this duct. For the purposes of this study, we have considered the duct to be homologous with the siphuncle.

Character No. 2: Gladius ostracum. Character states: 0 - present; 1- absent.

Comments. The internal shell of many Recent coleoid cephalopods consists of a thin, chitinous, often feather-shaped structure called the gladius (Figs. 2, 3). Bizikov (1991) considered the gladius to consist of three parts: the periostracum (also known as the rostrum); the ostracum (usually the primary component and often the only obvious component of the gladius), and the hypostracum (a cartilage-like thickening of the gladius apparent in some species). Only the ostracum was considered for this character. The shell (cuttlebone) of sepiids looks very different from a gladius. However, if the calcareous material of this shell is dissolved away, a chitinous structure resembling a broad gladius ostracum remains (along with other separable components) (Fig. 2B). We assume this to be the homologue of the gladius. This is not the case in *Spirula* where only components of the septa and siphuncle remain after dissolution of the calcium carbonate. In some other groups (Sepiolidae, Idiosepiidae) the ostracum is reduced in length but this was not considered as a separate character state at the present level of analysis. The cartilage-like shell of the cirrate octopods is superficially, at least, similar to the "hypostracum" of some teuthoids, and we do not consider this or the stylets (shell remnant of some incirrates) to be "ostraca" in the sense employed here.

Character No. 3: Shell composition. Character states: 0 - calcareous material present; 1 - chitin without calcareous material; 2 - without "typical" chitin or calcareous material (*i.e.* cartilaginous); 3- shell absent.

Comments. In most cases the shell composition in this study was estimated just from the visual appearance of the shell. A white, chalky shell as seen in *Nautilus, Sepia,* and *Spirula* was coded as calcareous; a thin, generally amber shell as seen in teuthoids was coded as chitinous; a thick, translucent structure as seen in cirrate octopods was coded as cartilaginous. Octopod stylets were classified as state 2 rather than as a fourth state. Chemical analysis would probably better define these states.

Character No. 4: Internal shell shape. Character states: 0 - flat and elongate; 1 - U-shaped; 2 - coiled; 3 - stylets; 4 -

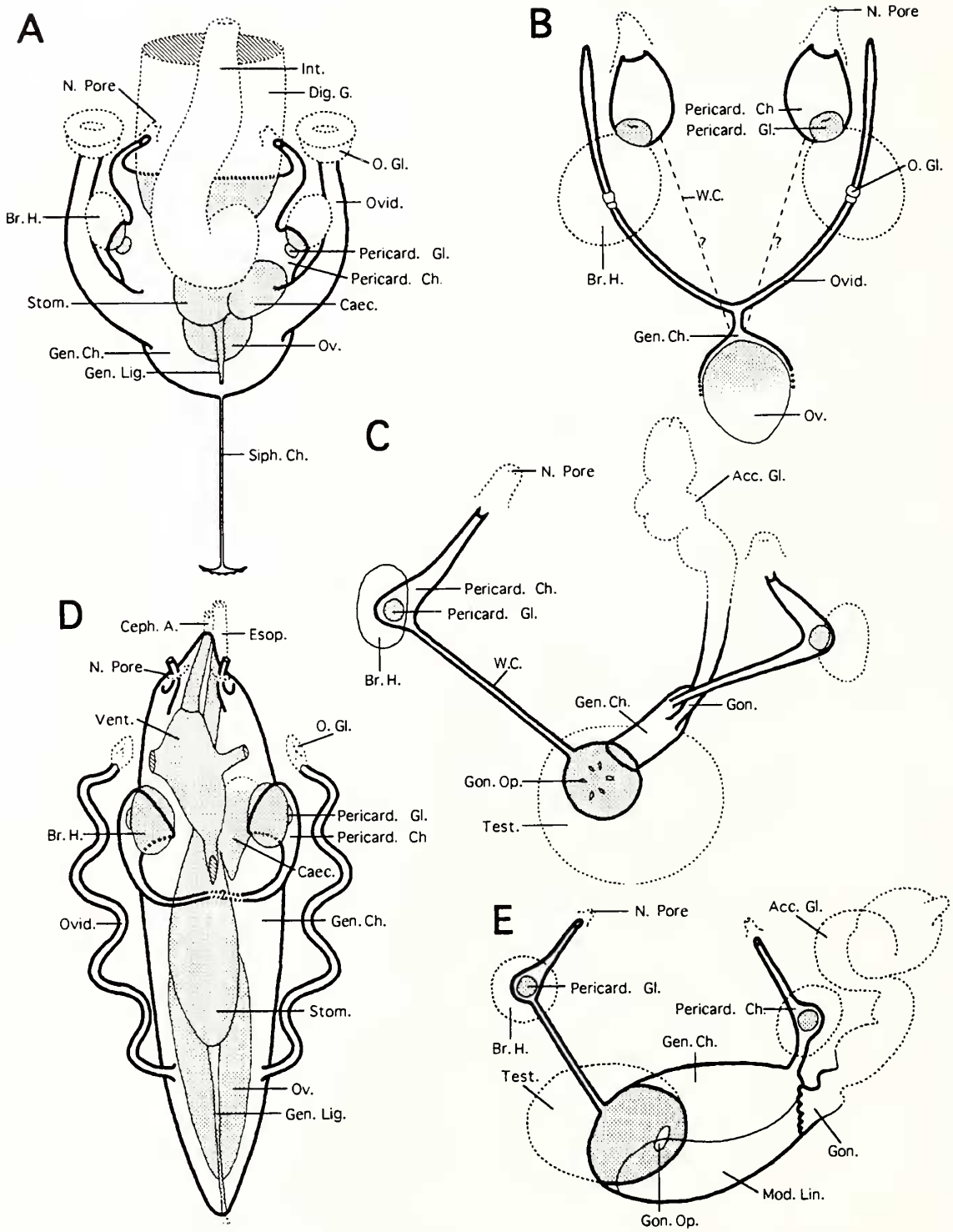

Fig. 1. Visceropericardial coelom. A. *Vampyroteuthis*. B. *Japetella*. C. *Grimpoteuthis*. D. *Sthenoteuthis*, shaded portions of the ink sac, branchial hearts, and intestine are covered by coelomic lining (*i.e.* they "lie" within the coelomic cavity). E. *Stauroteuthis*. (Acc. Gl., accessory gland; Br. H., branchial heart; Caec., caecum; Ceph. A., cephalic artery; Dig. G., digestive gland; Esop., esophagus; Gen. Ch., genital chamber of coelom; Gen. Lig., genital ligament; Gon., gonoduct; Gon. Op., gonadal opening to coelom; Int., intestine; Mod. Lin., modified lining; N. Pore, nephridial pore; O. Gl., oviducal gland; Ov., ovary; Ovid., oviduct; Pericar. Ch., pericardial chamber of coelom; Pericar. Gl., pericardial gland; Siph. Ch., siphuncular chamber of coelom; Stom., stomach; Test., testis; Vent., ventricle; W.C., water canal, thickness exaggerated).

NA (absent).
Comments. This character refers to the general shape of the internal shell. "Elongate" refers to elongation in the direction of the body axis (Fig. 3A). Stylets are elongate but not strictly in the direction of the body axis (Fig. 10B) and are not flattened. NA (not applicable) refers to the loss of an internal shell in some incirrate octopods. Under "U-shaped" we included the various modifications found in cirrate octopods (see Voss, 1988).

Character No. 5: Buccal crown. Character states: 0 - absent; 1 - present as oral arms; 2 - present.
Comments. The buccal crown consists of the muscular buccal supports and the connecting membrane that surrounds the mouth and lips (Fig. 4A). Naef (1928) considered the oral arms (inner ring of tentacles) of *Nautilus* (Fig. 4B) to be homologous with the buccal crown of decapods because of their similar location and because the buccal supports in decapods arise from secondary budding off the arm buds (in Boletzky, 1993b). Also, the buccal supports resemble arms in their possession of suckers in a few families (Fig. 4A). We, therefore, consider the presence of a buccal crown to be plesiomorphic within the Coleoidea.

Character No. 6: Arms II. Character states: 0 - unmodified; 1- filaments; 2 - absent.
Comments. The presence of ten equal arms in early coleoid fossils (*e. g. Jeletzkya*, Middle Pennsylvanian) suggests that the ancestral coleoid had ten equal arms. Naef (1928) noted that in the developing octopod embryo the primary folds from which the eyelids derive lie between arms II and III while in the decapods they lie between arms III and IV suggesting that the missing arm in octopods was one of the first three pairs (in Boletzky, 1993a). Because the first arm rudiment is widely separated from the others only in decapods, he suggested that the first arms were missing in octopods. Boletzky (1978-1979), however, found another marker, the metabrachial vesicles, that lies between arms I and II in both octopods and decapods and suggested that the missing arms are either arms II or III. The filaments in *Vampyroteuthis* lie in the position of a second pair of arms (Fig. 3A). R. E. Young (1967) found that the primary nerve trunks between the filaments and the subesophageal lobes of the brain largely bypassed the brachial lobe and suggested that the filaments might be homologous with the pre-ocular tentacles of *Nautilus*. On the other hand, J. Z. Young (1977) found, by dissection, nerves extending from the filament to each of two ganglia

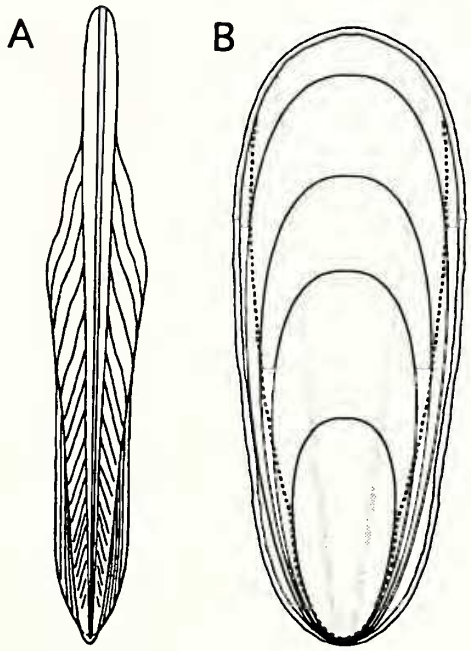

Fig. 2. Gladius ostracum. A. *Abralia*. B. *Sepia*, extracted from cuttlebone by dissolving calcium carbonate in weak acid. Ovals are representative growth lines; oblique lines mark inner margins of thickened layers on the ventral surface of the ostracum.

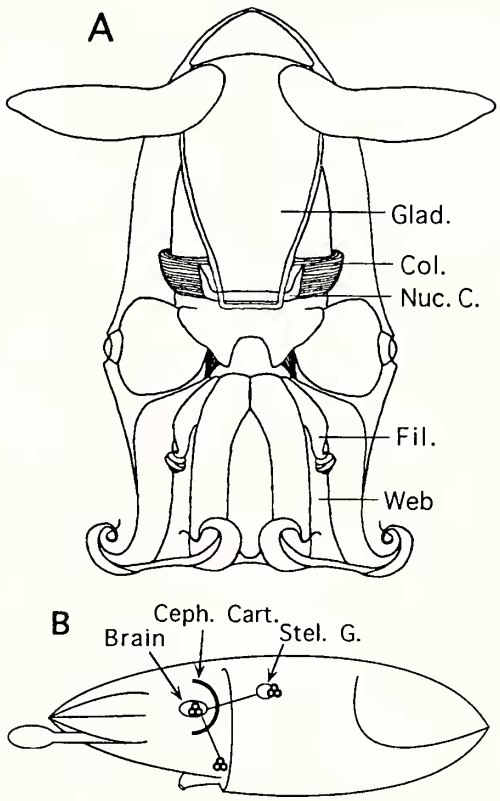

Fig. 3. A. Diagrammatic illustration of *Vampyroteuthis* showing position of the filaments, nuchal cartilage and gladius (modified after R. E. Young, 1962). B. Generalized cephalopod showing positions occupied by photosensitive vesicles. (Ceph. Cart., cephalic cartilage; Col., collar muscle; Fil., filament; Glad., gladius; Nuc. C., nuchal cartilage; Stel. G., stellate ganglion).

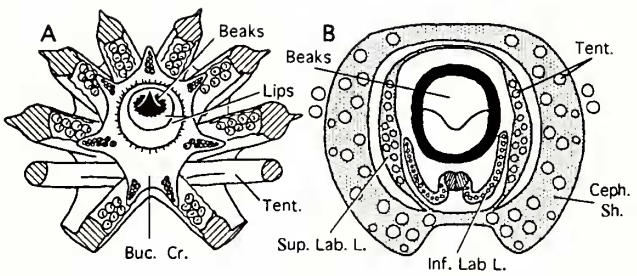

Fig. 4. Oral view of arm crowns (diagrammatic). A. *Loligo* (after Naef, 1921-1923) showing armature of arms in two series and suckers on the buccal crown. B. *Nautilus* (modified after Griffin, 1900). (Buc. Cr., buccal crown; Ceph. Sh., cephalic sheath; Inf. Lab. L., inferior labial lobe bearing tentacles; Sup. Lab. L., superior labial lobe bearing tentacles; Tent., tentacle).

on the circumoral commissure (= nerve ring). The latter connects the axial nerves of all the arms. He considered this proof that the filaments were modified arms II. We have made several attempts to confirm the existence of these connecting nerves to the filaments but failed to find them. Numerous small nerves radiate from the ganglia on the circumoral commissure to various muscles. In addition, the region is crossed by numerous slender muscle or connective tissue fibers. As a result, the nervous connections could be easily misinterpreted. The possible absence of a connecting nerve again opens the question of the homology of the filaments but does not disprove their origin from the second pair of arms. Indeed, the axial nerve of the tentacles (fourth arms) of some decapods (*e. g. Loligo, Sepia*) does not have a connection with the circumoral ring (Fig. 5B).

For this study, character coding was based on the assumptions that (1) the octopods have lost arms II, and that (2) the vampire filaments are modified arms II. Under these assumptions, the character states can be analyzed as "ordered": unmodified - filaments - absent.

Character No. 7: Arms IV. Character states: 0 - unmodified; 1 - tentacles.
Comments. Decapods retain the ten arms of the ancestral coleoid but arms IV have been modified into tentacles (Fig. 4A).

Character No. 8: Sucker rings. Character states: 0 - cuticular rings; 1 - no rings; 2 - horny rings.
Comments. This character refers to the secreted linings of suckers. The cuticular lining in octopods is chitinous (Hunt

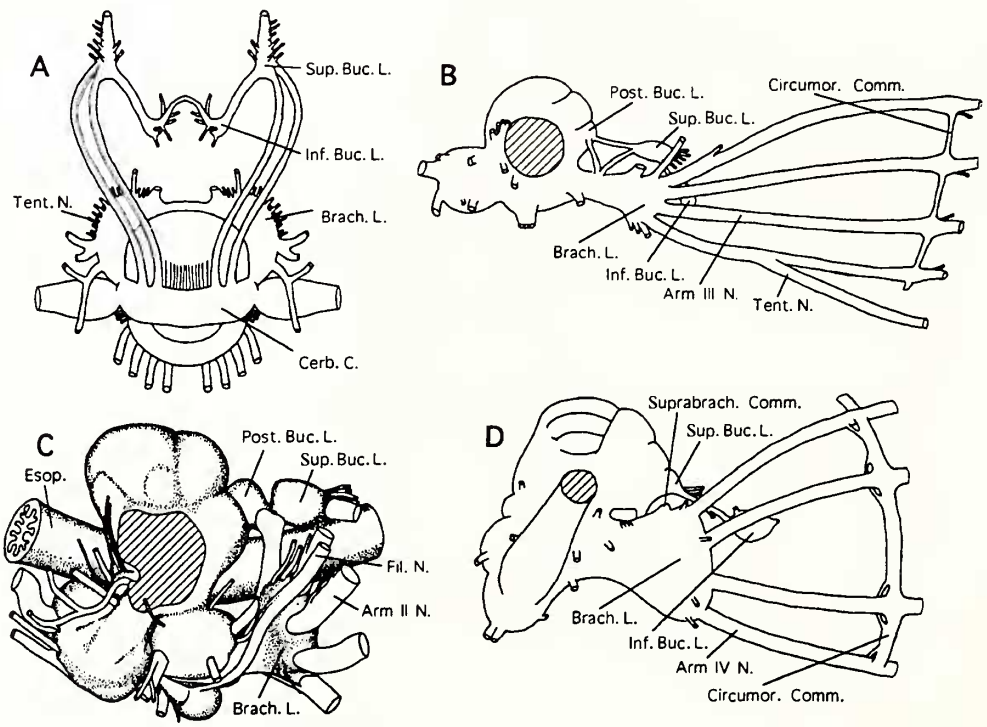

Fig. 5. Central nervous system (brain). A. *Nautilus* (modified after Griffin, 1900). B. *Sepia* (modified after Hillig, 1913). C. *Vampyroteuthis* (modified after R. E. Young, 1967). D. *Octopus* (modified after J. Z. Young, 1971). Arm numbering between taxa is based on morphological position not necessarily homology. (Arm II N., arm II axial nerve; Arm III N., arm III axial nerve; Arm IV N., arm IV axial nerve; Brach. L., brachial lobe; Cerb. C., cerebral cord; Circumor. Comm., circumoral commissure; Esop., esophagus; Fil. N., filament axial nerve; Inf. Buc. L., inferior buccal lobe; Post. Buc. L., posterior buccal lobe; Sup. Buc. L., superior buccal lobe; Suprabrach. Comm., suprabrachial commissure; Tent. N., tentacle nerve).

and Nixon, 1981). The inner "chitinous" sucker rings found in decapods are thick, generally bear teeth, and are more properly termed "horny" rings because they do not contain chitin (at least in *Sepia*; Rudall, 1955). Otherwise, the chemical composition of the decapod rings is unknown (Nixon and Dilly, 1977). Nixon found no trace of a cuticular lining in *Vampyroteuthis* and our sections confirm her observations. Neither we nor Nixon, however, had suckers in perfect condition.

Character No. 9: Sucker stalks. Character states: 0 - base and neck; 1 - base and plug; 2 - cylinder.
Comments. In octopods, a sucker stalk consists of a broad cylinder of muscles that attaches to the outer lateral walls of the acetabulum often at the point where the latter joins the infundibulum (Fig. 6A). Also, oblique muscle fibers cross within the cylinder (Graziadei, 1971). In decapods, a sucker stalk consists of a broad conical base that tapers gradually or abruptly to a neck of varying length (Fig. 6B). The neck is a narrow muscular rod that attaches off-center within the base of the sucker acetabulum. Superficially the sucker stalks in *Vampyroteuthis* resemble those of the decapods in having a broad base and a short neck (Fig. 6C). Both components, however, differ substantially from the decapod condition. The neck attaches (virtually adhears) to the connective tissue at the base of the acetabulum. In decapods the neck muscles and other tissues invade and form part of the acetabulum. The base in *Vampryoteuthis* does not clearly attach as a unit to the arm muscles. The major attachment is a band of muscles that runs proximally from the sucker neck, along the midline of the arm, to attach to the arm muscles beneath the base of the preceeding sucker. The relationships of the peculiar sucker stalk of *Vampyroteuthis* to that of the decapods and octopods is not clear at present and we have considered it a separate character state.

Character No. 10: Sucker symmetry. Character states: 0 - radial symmetry; 1 - bilateral symmetry.
Comments. The sucker is radially symmetrical in *Vampyroteuthis* and the octopods but strongly bilateral in the decapods (Fig. 6). The bilaterality of the latter is most apparent in the shape of the horny rings and the point of attachment to the stalk.

Character No. 11: Arm III armature series. Character states: 0 - one; 1 - two; 2 - four; 3 - more than four.
Comments. "Armature series" refers to the number of sucker and/or hook series paralleling the arm axis in the midportion of the arm (Fig. 4A). We restricted the character to the arm midportion as the armature series often differs at the tips and bases of the arms. In incirrate octopods, suckers almost invariably begin in embryos as a single series regardless of the number of series in the adults (Naef, 1921-1923). This suggests that a single series is plesiomorphic in the Octopoda. In decapods, hatchlings of many species have two or more rows and the plesiomorphic state is uncertain. *Nautilus* has a series of rings encircling the arms that can form a suction on their oral surfaces. This could be interpreted as a precursor to a single sucker series in early coleoids. This possibility, however, is contradicted by the presence of two rows of hooks in one of the oldest coleoids (*Jeletzkya*).

Character No. 12: Arm V sucker series. Character states: 0 - one; 1- two; 2 - four; 3 - more than four.
Comments. Armature series on arms V is not always the same as on arms III. As a result we regarded this as a separate character. As above the armature series refers to that in midarm.

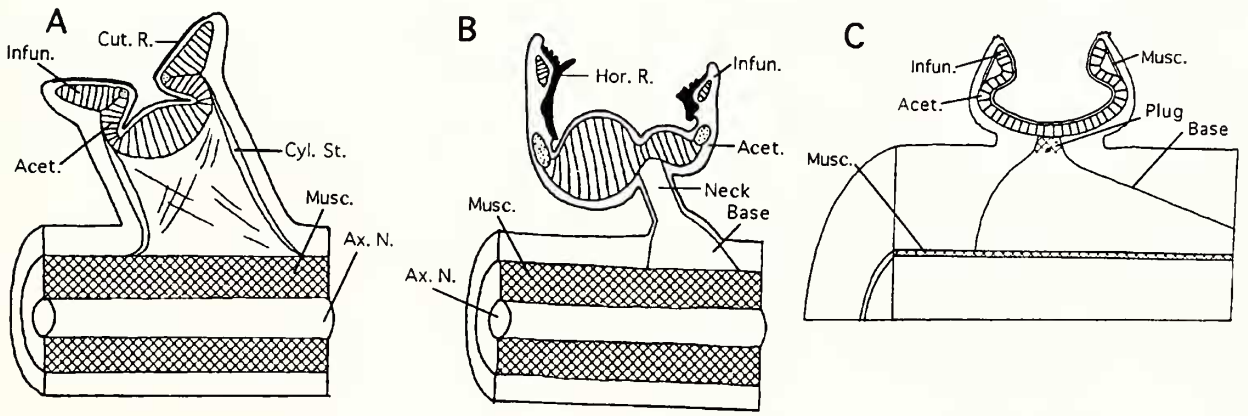

Fig. 6. Longitudinal sections of suckers (diagrammatic). A. *Octopus* (modified after Naef, 1921-1923). B. *Loligo* (modified after Naef, 1921-1923). C. *Vampyroteuthis*. (Acet., acetabulum; Ax. N., axial nerve; Cut. R., cuticular ring; Cyl. St., cylindrical stalk; Hor. R., horny ring; Infun., infundibulum; Musc., muscle).

Character No. 13: Trabeculae on unmodified arms. Character states: 0 - present; 1 - absent.
Comments. "Unmodified" refers to arms not modified for sexual functions. Trabeculae, in their most recognizable form, are conical, muscular structures that arise lateral to the suckers on the arms and are attached to the muscular cylinder of an arm. In some cases the identification of trabeculae is difficult without sectioning. Our character states were coded on the basis of general appearance and not histological sections. We, therefore, defined trabeculae as "present" only when they were clearly recognizable from their associated membranes. Therefore, if trabeculae are present but obscure, they will be incorrectly coded as absent. When membranes are lacking, possible trabeculae can be confused with skin folds associated with sucker bases. In this case, a distinct muscular pillar must be recognized to qualify as a trabecula. Trabeculae are not to be confused with the muscular sucker bases with which they are often associated. The origin of trabeculae is uncertain but they are similar in structure to sucker bases and, indeed, in a variety of cephalopods sucker bases are modified into structures apparently identical with trabeculae (*e. g.* on hectocotylized arms in a variety of squids, on the distal tips of the arms in *Vampyroteuthis*). Although the trabeculae are modified in a variety of ways in decapods, in many (*e. g. Thysanoteuthis*) the basic structure and attachment are identical to the cirri of octopods. Naef (1921-1923) considered trabeculae and cirri to be homologues and they were treated so in this study.

Character No. 14: Protective membranes on unmodified arms. Character states: 0 - absent; 1 - present.
Comments. "Unmodified" refers to arms not modified for sexual functions. An arm protective membrane is a membrane that connects trabeculae and lies lateral to the suckers/hooks. When trabeculae cannot be clearly recognized, the definition changes to: a membrane that is uninterrupted, at least along its free margin, by sucker bases. The octopod, *Ocythoe*, for example has membranes that are completely interrupted by suckers (*i. e.* the membrane extends from sucker to sucker) and, therefore do not qualify as "protective membranes" as defined here. To code this character as present, membranes need be present only on a portion of an arm, *e. g. Vampyroteuthis* which has protective membranes only near the arm tips (Pickford, 1946). There is no relevant character state for *Nautilus*.

Character No. 15: Well-developed interbrachial web between arms I. Character states: 0 - virtually absent; 1 - present.
Comments. "Well-developed" means the center of the web extends more than 20% of the arm length measured from the point where adjacent arms join (Fig. 3A). The sector between arms I was picked because this sector often has the lowest web development among the dorsal arms. Coding of this character was somewhat subjective in a few cases.

Character No. 16: Well-developed interbrachial web between arms V. Character states: 0 - absent; 1 - present.
Comments. "Well-developed" means the center of the web extends more than 20% of the arm length. The sector between arms V was picked because this sector generally has the lowest web development among the ventral arms. The absence of a web between arms V is often independent of the web condition of the dorsal arms. *Nautilus* lacks a relevant character state.

Character No. 17: Fin cartilage. Character states: 0 - at proximal end of fin; 2 - at proximal end and in core; 3 - NA (fins absent).
Comments. In decapods the fins typically insert on a flattened cartilage with a slight medial ridge; the cartilage attaches to the shell sac. In cirrates the fin cartilage has generally been considered to be absent (Robson, 1932). In *Vampyroteuthis* the problem of comparison is complicated by the fact that this animal has two pairs of fins. The juvenile fin appears first and the adult fin later in a more anterior position; with growth the juvenile fins are resorbed and the adult fins enlarge (Pickford, 1950). In dissecting and sectioning the fins of cirrates, we found that they have a fin cartilage with a small "base" that adheres to the shell sac and an extensive "plate" that occupies the core of the proximal half of the fin (Figs. 7A-D). The cartilage consists of a highly vacuolated tissue with virtually no matrix other than the thin walls of the vacuoles. The flexible, spongy consistency is apparently responsible for it not being previously recognized as cartilage. The adult vampire fin has a small cartilage at the tip of the attached end of the fin and the fin has an L-shape that is very different from those of cirrates or decapods (Figs. 7E, F). The juvenile fin of *Vampyroteuthis* has an internal cartilage similar to that of the cirrates but with a much larger flattened, cartilaginous "base" attached to the shell sac and a slender cartilaginous "plate" (continuous with the base) that extends through the entire core of the fin (Figs. 7G, H). The histology is the same as in the cirrates except that the vesicles are larger in *Vampyroteuthis* (Fig. 7I). We consider the juvenile fin of *Vampyroteuthis* to be the homologue of the octopod and decapod fins.

The absence of fins in incirrates is clearly secondary as their anlagen are present in the embryo but disappear during development (Naef, 1921-1923).

Character No. 18: Statocyst outer capsule. Character states: 0 - outer capsule absent; 1 - outer capsule present.
Comments. Statocysts have one of two basic structures in

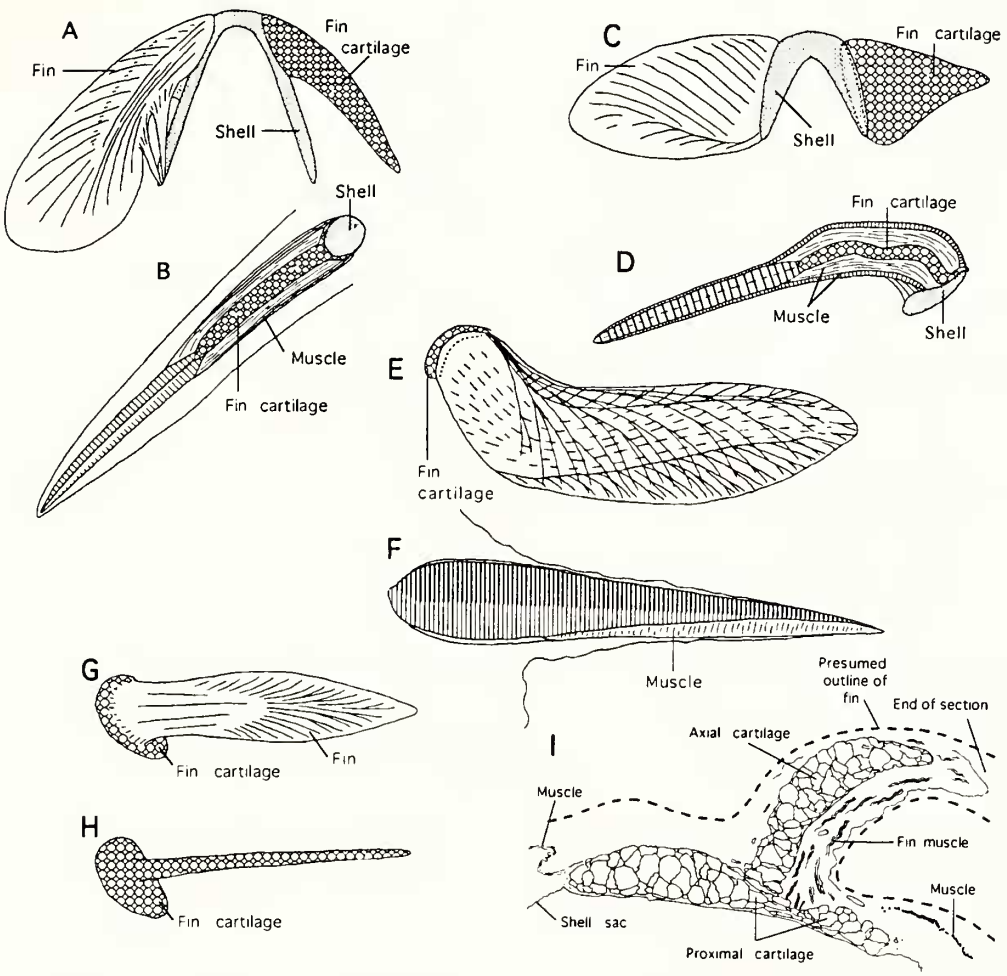

Fig. 7. Fins (diagrammatic). A. *Grimpoteuthis*, ventral view with outer tissue removed. B. *Grimpoteuthis*, longitudinal section through fin. C. *Stauroteuthis*, ventral view with outer tissue removed. D. *Stauroteuthis*, longitudial section through fin. E. *Vampyroteuthis*, adult fin, dorsal view. F. *Vampyroteuthis*, adult fin, longitudinal section through straight portion of fin. G. *Vampyroteuthis*, juvenile (= "larval") fin, dorsal view. H. *Vampyroteuthis*, juvenile fin, dorsal view with outer tissue removed. I. *Vampyroteuthis*, longitudinal section through juvenile fin.

cephalopods: they consist of a single sac that is commonly embedded directly in cartilage or they lie within an additional fluid-filled sac, the outer capsule, which is commonly embedded in cartilage.

Character No. 19: Nephridial coelom. Character states: 0 - nephridial coeloms separate (unipaired); 1 - nephridial coeloms fused (single coelom).
Comments. In *Vampyroteuthis infernalis*, left and right nephridial sacs are separated from one another by their medial walls (Fig. 8A) and further by the intestine which lies between the medial walls at their ventral ends (Fig. 8B). Each has a rather simple shape and includes renal appendages arising from (1) the cephalic vein, (2) vena cava en route to the branchial heart, and (3) a dorsal branch of the vena cava (the latter forms an abbreviated dorsal lobe).

In the incirrate *Japetella diaphana* the left and right nephridial sacs are, also, entirely separate and a more distinct dorsal lobe is present (Figs. 8C, D).

The nephridial coelom has not been well described in the teuthoids. In dissecting *Sthenoteuthis oualaniensis* (Lesson, 1830) we found the coelom divided into two chambers: the dorsal and ventral sacs (Figs. 8E, F). The ventral sac is roughly Y-shaped with the stem of the Y directed anteriorly and the arms defined by a septum that partially divides the cavity posteriorly. The cephalic vein enters the cavity dorsally. The renal appendages are restricted to the ventral sac although the cephalic vein has what seems to be tiny appendages near its dorsal entrance. The ventral sacs surround the intestine, ink sac, and the extensive lobes of the digestive gland duct appendages (DGDA) (see Character 43). Communication between the dorsal and ventral sacs occurs to either side of the inflated

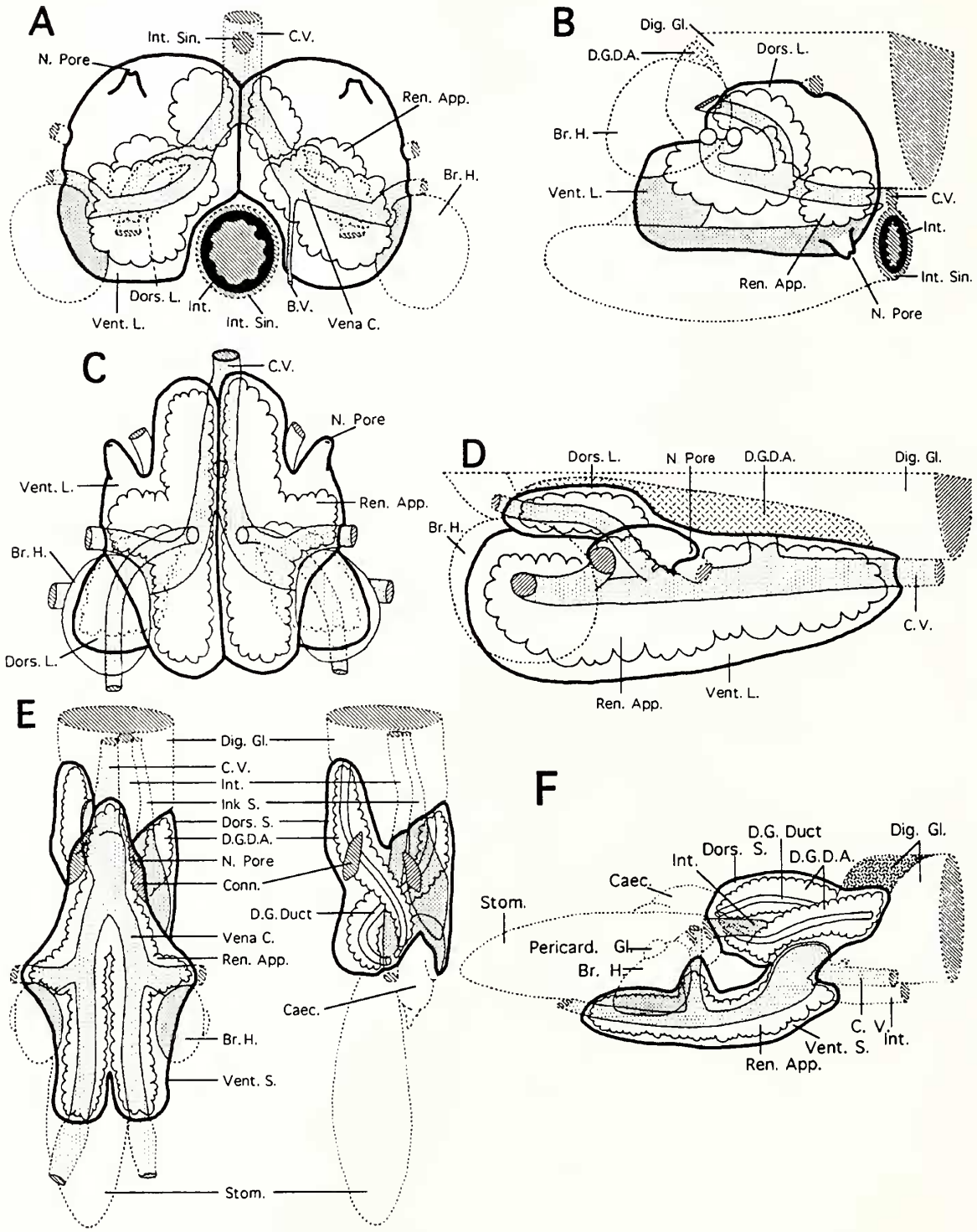

Fig. 8. Nephridial coelom (diagrammatic). A. *Vampyroteuthis*, ventral view. B. *Vampyroteuthis*, side view. C. *Japetella*, ventral view. D. *Japetella*, side view. E. *Sthenoteuthis*, left: ventral view; right: ventral view with ventral sac removed. F. *Sthenoteuthis*, side view. (B. V., blood vessel; Br. H., branchial heart; Caec., caecum; Conn., connection between dorsal and ventral sacs of coelom; C. V., cephalic vein; D. G. D. A., digestive gland duct appendages; D. G.Duct, digestive gland duct; Dig. G., digestive gland; Dors. L., dorsal lobe of coelom; Dors. S., dorsal sac of coelom; Ink S., ink sac; Int., intestine; Int. Sin., intestinal sinus; N. Pore, nephridial pore; Pericard. G., pericardial gland; Ren. App., renal appendages; Stom., stomach; Vena C., vena cava; Vent. L., ventral lobe of coelom; Vent. S., ventral sac of coelom).

cephalic vein as it enters the ventral sac. This is generally the same as in *Sepia* (Tompsett, 1939).

In the cirrate *Grimpoteuthis glacialis* (Robson, 1930) the nephridial sacs are also paired (Fig. 9A). Each sac has a very extensive dorsal lobe. The right sac has a dorsal branch that extends posteriorly as a large sac that circles the gonad to its posterior tip and contains renal appendages throughout most of its course. The dorsal branch of the left sac also has a posterior extension but it is very broad and lacks renal appendages although they are present dorsally.

In the cirrate *Stauroteuthis syrtensis* the nephridial sacs are paired as well (Fig. 9B). The dorsal branch off the vena cava is longer than in *Japetella* and carries the bulk of the renal appendages. The dorsal lobe, therefore, is large but its full extent was not mapped.

This character can be difficult to evaluate in preserved specimens. In general if the renal appendages had a Y-shaped morphology along their ventral face (*i. e.* renal appendages begin on the cephalic vein), we assumed that the coelom of either side was continuous (fused) medially.

Character No. 20: Visceropericardial coelom. Character states: 0 - extensive coelom (surrounds visceral nucleus, ventricle and gonad); 1 - coelom reduced (viscera excluded except part of the gonad).

Comments. We have illustrated the visceropericardial (VP) coelom of several species which have not been illustrated previously. The VP coelom of *Vampyroteuthis* (Fig. 1A) surrounds most of the visceral nucleus, much of the posterior end of the digestive gland including part of the digestive gland duct appendages (but see Character 43), posterior end of the crop, ventricle, and the gonad which is suspended in the coelom from the genital strand. The pericardial chamber is represented by short outpocketings on either side of the coelom that enclose the pericardial glands and the medial portions of each branchial heart. At its antero-lateral corners the coelom narrows into ducts that open into the nephridial coelom at the base of the nephridial papillae.

In *Japetella diaphana* the VP coelom (Fig. 1B) is very restricted in extent as appears typical of the incirrate octopods (see Isgrove, 1909). We were, however, unable to locate the water canals but they could have been missed. The degree to which the gonad lies in the coelom could not be fully evaluated as the tissue layers did not separate cleanly. However, the ventral half of the gonad and part of the anterior portion appeared to be within the coelomic cavity.

In *Grimpoteuthis glacialis* the VP coelom is very restricted but less so than in *Japetella* (Fig. 1C). It covers just a small patch of the gonad (here the gonad opens into the coelom). This portion is separated from the rest of the coelomic sac (the outer sac) by a transverse membrane that

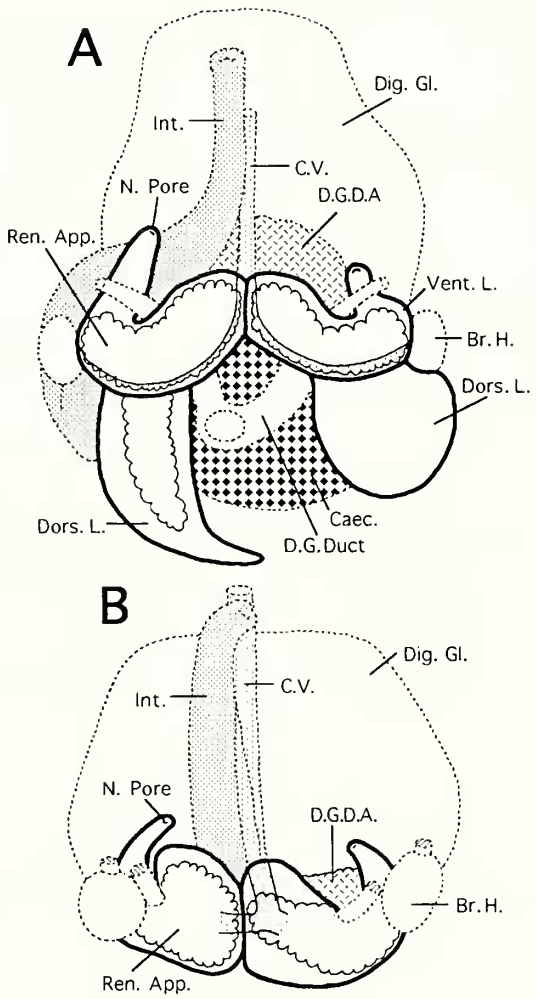

Fig. 9. Nephridial coelom (ventral view, diagrammatic). A. *Grimpoteuthis*. B. *Stauroteuthis*. (Br. H., branchial heart; C. V., cephalic vein; Caec., caecum; D. G. D. A., digestive gland duct appendages; D. G. Duct, digestive gland duct; Dig. G., digestive gland; Dors. L., dorsal lobe of coelom; Int., intestine; N. Pore, nephridial pore; Ren. App., renal appendages; Vent. L., ventral lobe of coelom).

partially occludes the connection. The gonoduct and the left water canal open into the outer sac near its lateral extent and the right water canal opens into the inner sac. Both water canals extend to a small sac covering the pericardial gland. Anterior ducts from the latter sacs open into the nephridial coelom at the base of the nephridial papillae. There appears to be no genital pocket in the male.

The VP coelom of *Sthenoteuthis oualaniensis* is generally representative of the decapods (Fig. 1D). It extends from the digestive gland to the conus of the gladius and incorporates most of the visceral nucleus (stomach and caecum), ventricle, posterior esophagus, and the gonad. The pericardial chamber of the VP coelom consists of a shelf on the ventral surface of the coelom and encloses

much of the branchial hearts and pericardial glands and appears to be continuous across the ventral midline although we could not be certain of this. Anteriorly the VP coelom narrows abruptly into ducts which extend through the nephridial pores to open into the mantle cavity. The extension of the ducts into the mantle cavity could be a peculiarity of this species.

In *Staurotеuthis syrtensis*, the VP coelom is less restricted than in *Grimpoteuthis* and is reduced mostly to a sac that covers, at its right end, a portion of the gonad and leads, at its left end, into the large fluted end of the broad male gonoduct (Fig. 1E). Two narrow ducts ("water canals") extend from the coelomic sac to include the pericardial glands. The right duct is long and slender and the left one short and somewhat broader (the thickness of both is exaggerated in the illustration). From the sac around the pericardial gland a broader and more muscular duct opens into the nephridial coelom at the base of the nephridial papilla on either side. There appears to be no genital pocket in the male.

In *Nautilus*, the VP coelom is extensive and divided into pericardial, genital, and siphuncular chambers. The gonad, part of the intestine, stomach, digestive gland, and ventricle are covered by coelomic epithelium (Griffin, 1900).

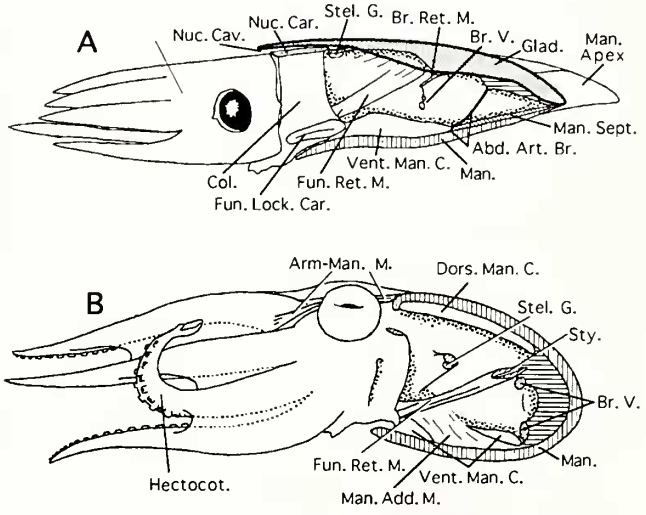

Fig. 10. The teuthoid *Abralia* showing the mantle septum, nuchal cartilage, nuchal cavity, funnel-locking cartilage, and position of stellate ganglion. Heavy line outlines gladius. B. An incirrate octopodid showing the dorsal mantle cavity, position of stellate ganglion, muscle connection between arm bases and anterior mantle margin, mantle adductor muscle, stylets, and hectocotylus. [Abd. Art. Br., abdominal (median pallial) artery and branch (lateral pallial artery); Arm-Man. M., arm-mantle muscle; Br. Ret. M., branchial retractor muscle; Br. V., branchial blood vessel; Col., collar; Dors. Man. C., dorsal mantle cavity; Fun. Lock. Car., funnel locking cartilage; Fun. Ret. M., funnel retractor muscle; Glad., gladius; Hectocot., hectocotylus; Man., mantle; Man. Add. M., mantle adductor muscle; Man. Apex, mantle apex; Man. Sept., mantle septum; Nuc. Car., nuchal cartilage; Nuc. Cav., nuchal cavity; Stel. G., stellate ganglion; Sty., stylet; Vent. Man. C., ventral mantle cavity].

Character No. 21: Dorsal mantle cavity. Character states: 0 - absent; 1 - present.
Comments. This cavity is a dorsal continuation of the ventral mantle cavity, across the dorsal midline, posterior to the stellate ganglia (Fig. 10B). The large dorsal cavity of *Spirula* is excluded from state 1 by its presence anterior to the stellate ganglia and we consider it to be a "nuchal" cavity. Because *Nautilus* lacks stellate ganglia, we define the dorsal mantle cavity, in this case, as a cavity well posterior to the level of the collar. The cavity in the collar region, the "nuchal" cavity, is characteristic of most cephalopods in which the mantle and head are not fused (Fig. 10A). The dorsal mantle cavity as defined here proved to be characteristic of the octopods.

Character No. 22: Nidamental glands. Character states: 0 - absent; 1 - present.
Comments. Nidamental glands of decapods are large, paired organs that open directly into the mantle cavity and are composed of numerous lamellae that are involved in secretion of egg cases or masses. *Nautilus* has a three-lobed, lamellar nidamental gland.

Character No. 23: Crop. Character states: 0 - present; 1 - absent.
Comments. The crop is an expansion or a diverticulum of the esophagus for food storage. For this study, we consider a crop to be absent unless it is morphologically obvious. The cirrate octopods we examined have an esophagus that is only slightly expanded and we considered this as state 1. Others (see Robson, 1932) have indicated the presence of a reduced crop in cirrates which could simply reflect differences in how a crop is defined. These statements, nevertheless, have resulted in our coding the cirrates as polymorphic for this character.

Character No. 24: Branchial canal. Character states: 0 - absent; 1 - present.
Comments. The branchial canal is a large opening at the base of each gill lamella and between the primary afferent and efferent blood vessels of the gill (Figs. 11A, D, E). This canal allows passage of sea water between lamellae at this point. This unambiguous character was a primary feature used by systematists for many years to separate teuthoids from sepioids.

Character No. 25: Mantle septum. Character states: 0 - absent; 1 - present and continuous; 2 - present but open posteriorly; 3 - blood vessel only.

Comments. The mantle septum passes from the ventral surface of the visceral mass, across the mantle cavity to the inner surface of the mantle wall (Fig. 10A). The membrane lies in an anterior/posterior orientation and divides the mantle cavity into right and left sides. Along its anterior margin, the membrane supports, in decapods, a branch of the abdominal aorta as it passes from the visceral mass to the ventral mantle wall. Character state 3 occurs only in *Spirula* where the coiled shell leaves room for only the artery. No septum is present in *Nautilus*; however, the very different organization of the mantle cavity suggests that this character is not applicable to *Nautilus*.

Character No. 26: Mantle adductor. Character states: 0 - absent; 1 - present; 2 - NA.
Comments. The mantle septum commonly has slender muscle fibers running along it primarily in an anterior-posterior direction. In some cephalopods, a pronounced muscle bundle, the mantle adductor, is present that runs more or less ventrally from the visceral mass to the mantle wall (Fig. 10B). Presumably an adductor cannot exist without a mantle septum as a precursor. NA refers to the absence of the mantle septum. *Nautilus* lacks a relevant character state.

Character No. 27: Funnel valve. Character states: 0 - present; 1 - absent.
Comments. The funnel valve is a muscular flap, continuous with the postero-dorsal wall of the funnel, that lies within the lumen of the funnel.

Character No. 28: Nuchal cartilage. Character states: 0 - present; 1 - absent.
Comments. The nuchal cartilage is the support for the head component of the nuchal locking apparatus (Fig. 10A). Muscles of the collar and muscles from the head and shell sac attach to this cartilage. In *Vampyroteuthis*, although the head and mantle are fused, a nuchal cartilage remains (Fig. 3A). Its shape varies from a flat rectangle to a low U-shape and lies beneath and just posterior to the anterior end of the cartilage that surrounds much of the periphery of the gladius. The cartilage no longer supports a locking apparatus but still provides a site for muscle attachment. *Nautilus* lacks a mantle-propulsion system which, presumably, is a prerequisite for development of a nuchal cartilage; it has no relevant character state.

Character No. 29: Cornea. Character states: 0 - cornea absent; 1 - one-part cornea present; 2 - two part cornea present.
Comments. The cornea is a transparent, protective covering of the lens. Here we consider all one-part corneas as homologues; although this is a debatable assumption it is not critical at this level of analysis. *Nautilus* lacks a relevant character state because its eyes do not bear lenses.

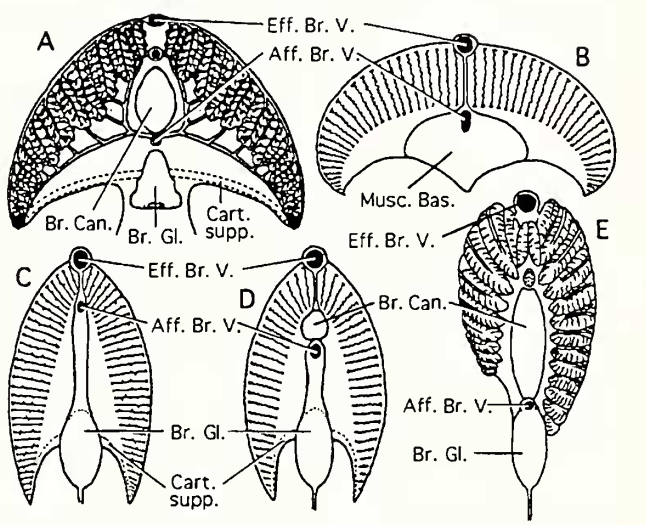

Fig. 11. Gill filaments (diagramatic). A. *Vampyroteuthis* (modified after R. E. Young, 1962); B. *Nautilus* (modified after Naef, 1921-1923); C. *Sepia* (modified after Naef, 1921-1923); D. *Loligo* (modified after Naef, 1921-1923); E. Incirrate octopod (modified after Naef, 1921-1923). (Aff. Br. V., afferent branchial vessel; Br. Can., branchial canal; Br. Gl., branchial gland; Cart. supp., cartilagenous supporting rod; Br. Gl., branchial gland; Eff. Br. V., efferent branchial vessel; Musc. Bas., muscular base).

Character No. 30: Right oviduct. Character states: 0 - absent; 1 - present.
Comments. The oviducts are gonoducts that open into the visceropericardial coelom and exit into the mantle cavity (Figs. 1A, B, D). In coleoids the left, but not the right, oviduct is always present. "Present" refers to physical presence irrespective of functionality.

Character No. 31: Oviducal gland symmetry. Character states: 0 - radial symmetry; 1 - bilateral symmetry; 2 - asymmetry.
Comments. The oviducal glands are organs that surround the oviducts and contain numerous glandular lamellae that are involved in secretion of egg cases or masses. The oviducal gland of *Vampyroteuthis* surrounds the oviducal opening and consists of two thick, contiguous, equal-sized rings. Each ring is composed of flattened lamellae. Typically the leaflets of the outer ring which are attached only proximally are freely exposed to the mantle cavity. A circular membrane that covers the more proximal ring, however, is capable of expanding to cover the outer ring. The entire organ is radially symmetrical (Fig. 12B). The double nature of the gland is characteristic of coleoids. In decapods the proximal portion is much smaller and both proximal and distal portions are bilaterally symmetrical (Fig. 12C) while in octopods the distal portion is often smaller and the glands

are radially symmetrical. The oviducal gland of *Nautilus* forms the terminal portion of the oviduct. It appears to be highly glandular and has thick folds or lamellae. The gland, in our poorly preserved specimens, has a slit-like opening and the arrangement of lamellae shows it to be asymmetrical (Fig. 12A).

Character No. 32: Oviducal gland position. Character states: 0 - gland terminal (located at end of oviduct); 1 - gland subterminal (oviduct continues distal to gland).
Comments. Oviducal glands are located either at the opening of the oviduct (Figs. 1A, D) or well proximal to the oviduct external opening which thereby defines a distinct distal oviduct (Fig. 1B). A distal oviduct can be identified by the lack of glandular leaflets and often lacks a circular orifice. In *Sepiola* the distal portion of the oviducal gland is very slender and elongate and gives the false impression of a distal oviduct.

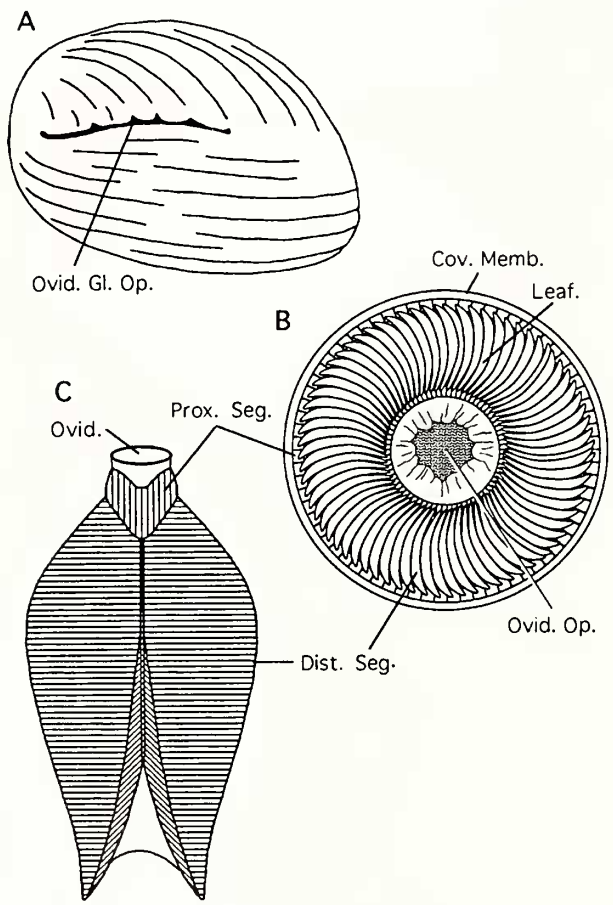

Fig. 12. Oviducal glands (diagramatic). A. *Nautilus*. B. *Vampyroteuthis*. C. *Abralia*. (Cov. Memb., covering membrane; Dist. Seg., distal segment of oviducal gland; Leaf., leaflet; Ovid., oviduct; Ovid. Gl. Op., oviducal gland opening; Ovid. Op., oviduct opening; Prox. Seg., proximal segment of oviducal gland).

Character No. 33: Photosensitive vesicles. Character states: 0 - within cephalic cartilage; 1 - above funnel; 2 - on stellate ganglia.
Comments. The photosensitive vesicles are vesicular organs that function in detection of light for a variety of purposes. In decapods they occupy a variety of locations but all are within the region of the head bounded by the cephalic cartilage (Fig. 3B). In octopods they lie on the stellate ganglia and in *Vampyroteuthis* they lie just above the dorsal surface of the funnel (Fig. 3B). Photosensitive vesicles have never been described in *Nautilus*. This is an unambiguous character that has been previously described in most families of concern here.

Character No. 34: Inferior frontal lobe system of the brain. Character states: 0 - absent; 1 - partially present; 2 - present.
Comments. The inferior frontal lobe system of octopods consists of the inferior frontal, subfrontal, and posterior buccal lobes (J. Z. Young, 1971). In decapods this entire system is represented by the posterior buccal lobes (often called the inferior frontal lobes) (J. Z. Young, 1988). In *Vampyroteuthis* the complex connections posteriorly from the posterior buccal lobe (*sensu lato*) and the central region of the supraesophageal mass is much more complex than in decapods and J. Z. Young (1977: 385) suggested it "may represent a poorly differentiated subfrontal lobe." He considered this and some differentiation of the dorsal part of the buccal lobe which he interpreted as an inferior frontal lobe, to be a stage of "incipient development of an apparatus for more elaborate processing of tactile information... ." We agree with this interpretation and coded this character as a separate state for *Vampyroteuthis* that is intermediate between the octopod and decapod conditions. This transformation series is best defined as "ordered." The construction of the brain of *Nautilus* is very different from coleoids and cannot be coded for this character.

Character No. 35: Head-mantle fusion: arm-base-to-anterior-mantle muscle. Character states: 0 - present; 1 - absent.
Comments. In a variety of coleoids the dorsal surface of the mantle has become fused to the head. The variation in the details of the fusion indicate that fusion has occurred several times during evolution. Character 35 describes a characteristic of the octopod fusion in which muscles attach at the junction of the dorsal arm bases and on the anterior edge of the dorsal mantle (Fig. 10B). As a result, it lumps all other families together under state 1 (absent). This is acceptable at our present level of analysis because state 1 is the plesiomorphic state in the Coleoidea. Even though *Nautilus* lacks a mantle-propulsion system, we consider its

lack of head-mantle fusion a relevant state.

Character No. 36: Arm III hectocotylization. Character states: 0 - absent; 1 - present.
Comments. Hectocotylization refers to the modification of an arm in males for the transfer of sperm to the female (Fig. 10B). Arm III in octopods and vampyromorphs could be the homologue of arm IV (tentacle) in decapods (see Character 6). For this character we have not made this assumption and compared the morphological arm III in all groups. The distribution of this character, however, would be the same for either interpretation of arm relationships. Either one or both members of an arm pair could be modified. Because *Nautilus* lacks specific homologues to each of the ten arms of coleoids, it has no relevant character state.

Character No. 37: Arm V hectocotylization. Character states: 0 - absent; 1 - present.
Comments. We assume that the hectocotylization of different arm pairs (cf. Character 36) represents independent evolutionary events and, therefore, qualify as separate characters.

Character No. 38: Collagenous tunics on mantle. Character states: 0 - absent; 1 - present.
Comments. Many decapods contain a collagenous tunic over the mantle that, apparently, acts to resist change in mantle length during the jet cycle (Ward and Wainwright, 1972). To evaluate this character, we have looked for a continuous connective tissue sheath over the inner and outer surfaces of the mantle muscle in histological sections. However, this approach did not always provide unambiguous results. Connective tissue is present on the mantle of all cephalopods and the difference between a continuous, uniform "tunic" and an irregular layer was not always clear. Indeed, we were unable to clearly distinguish what could be intermediate states in *Sepia* and *Octopus*. Because *Nautilus* lacks a mantle-propulsion system, a necessary precursor to the development of a collagenous tunic, it has no relevant character state.

Character No. 39: Spermatophores with ejaculatory apparatus. Character states: 0 - present; 1 - sperm packets; 2 - encapsulated coil.
Comments. All coleoid cephalopods except the finned octopods have characteristic spermatophores that contain a complex ejaculatory apparatus (Figs. 13A, B, D). The finned octopods produce peculiar sperm packets (Fig. 13E). This one character probably represents a suite of modifications to the spermatophore and the glands that form them, setting the cirrates apart from other coleoids. *Nautilus* has a spermatophore that lacks an ejaculatory apparatus

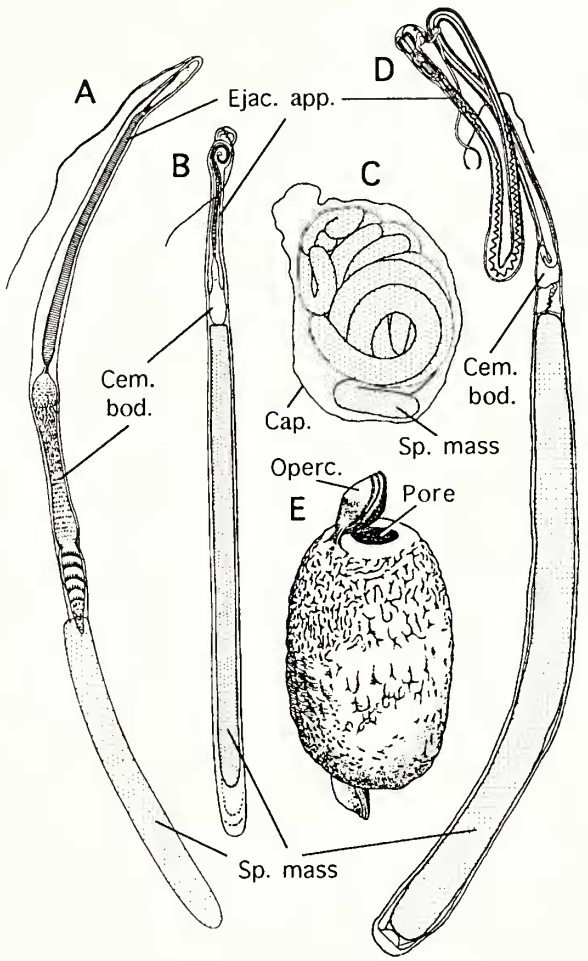

Fig. 13. Spermatophores. A. *Eledone* (modified after Marchand, 1912). B. *Loligo* (modified after Marchand, 1912). C. *Nautilus* (modified after Mikami and Okutani, 1981). D. *Vampyroteuthis* (modified after Hess, 1987). E. *Opisthoteuthis* (after Villanueva, 1992). (Cap., capsule; Cem. bod., cement body; Ejac. app., ejaculatory apparatus; Operc., operculum; Sp. mass, sperm mass).

(Mikami and Okutani, 1981), is very different from that of cirrate octopods (Fig. 13C), and has been given a separate character state (state 2).

Character No. 40: Superior buccal lobe. Character states: 0 - widely separated from brain; 1 - adjacent to brain; 2 - fused to brain.
Comments. The position of the superior buccal lobe relative to the supraesophageal mass of the brain varies greatly among different cephalopods depending largely on the distance between the buccal mass and the brain (Fig. 5). There are, however, three distinct states as defined above. State 0 is found in decapods and *Nautilus* (Figs. 5A, B), state 1 in *Vampyroteuthis* (Fig. 5C), and state 2 in octopods (Fig. 5D). J. Z. Young (1988: 255) misstated the condition in *Vampyroteuthis* as "the superior buccal lobes are joined

with the rest of the brain as in octopods" due to his examination of a distorted specimen (note the 90° turn in the esophagus in his fig. 5) that artificially compressed the superior buccal mass against the brain. In normal specimens the lobes are clearly separated medially. As a result we have given *Vampyroteuthis* a separate state which is intermediate between the decapod and octopod states and consider this character to have an "ordered" transformation series. A similar situation, with the exception of *Nautilus*, occurs with respect to the brachial lobe, but as this character is probably not independent from Character 40 in the coleoids, it was not used.

Character No. 41: Horizontal arm septa. Character states: 0 - absent; 1 - present.
Comments. Cirrate octopods have a horizontal septum that inserts on the circular muscle layer that forms the outer and thinner portion of the cyclindrical muscular wall of the arm. It is orally concave in cross-section and divides the muscular tube within each arm into oral and aboral regions. *Japetella* (Bolitaenidae) has a similar septum but its insertion on two membranes, extending in an oral-aboral plane well internal to the arm muscles, suggests that the cirrate and bolitaenid structures are derived independently.

Character No. 42: Paired digestive gland duct appendages. Character states: 0 - single; 1 - paired.
Comments. The digestive gland duct appendages (DGDA) are glands that attach to the ducts of the digestive glands. In decapods they are spread along the long ducts to form paired, multilobed structures (Figs. 8E, F) while in *Vampyroteuthis* and the octopods they are compacted next to the digestive gland in a single (unpaired) structure (Figs. 8B, D, 9). Although there is some variation in the Oegopsida, the organs always remain paired even where occasionally compacted against the digestive gland. Because *Nautilus* lacks DGDA, it has no relevant character state.

Character No. 43: Relative positions of the DGDA and the nephridial coelom. Character states: 0 - DGDA lies in the nephridial coelom; 1 - DGDA not in the nephridial coelom.
Comments. The DGDA can be suspended within a branch of the nephridial coelom and intimately covered by the coelomic lining (Figs. 8E, F) or lie outside the coelom and separated by, at least, several tissue layers from the coelom (Figs. 8B, D, 9). *Nautilus* lacks a relevant character state.

Character No. 44: Head width index. Character states: 0 - 0.49; 1 - 0.5-0.99; 2 - 1.0-1.49; 3 - 1.5-1.99; 4 - 2.0-2.49; 5 - 2.5-2.99; 6 - 3.0-3.49; 7 - 3.5-3.99; 8 - 4.0-4.49; 9 - 4.5-4.99.
Comments. This character relates head width to eye size by measuring the number of eye radii that separate the eyes. States were assessed by measuring the head width and the lens diameter. The eye radius was then calculated and doubled for subtraction from the head width. This is a continuous character that has been converted to a discrete character by dividing the range of the character into equal segments (Fig. 14). The definitions were compromised in groups (some sepioids and cirrate octopods) with a dorsal tilt to the eyes, and therefore, this character was excluded from the analysis.

Character No. 45: Gill filament attachment. Character states: 0 - inner and outer filaments free; 1 - outer filaments attached; 2 - inner and outer filaments attached.
Comments. In coleoids the gill filaments often are attached to the gill base surrounding the branchial gland by triangular membranes that are supported by slender cartilaginous rods (Figs. 11A, C, D). The length of the rod determines the distance of the filament from the branchial gland. The length of the rod varies with the position along the gill, the side of the gill, and the taxon. When the rod is absent in coleoids, the filament is defined as "attached" (Fig. 11E) and, in some cases, the difference between attached and free is slight. The gills of *Nautilus* are comparable to those of coleoids but lack a branchial gland and supporting rods to the filaments (Fig. 11B). The muscular triangular membranes broadly separate the tips of the filaments from the muscular base that occupies the position of the branchial

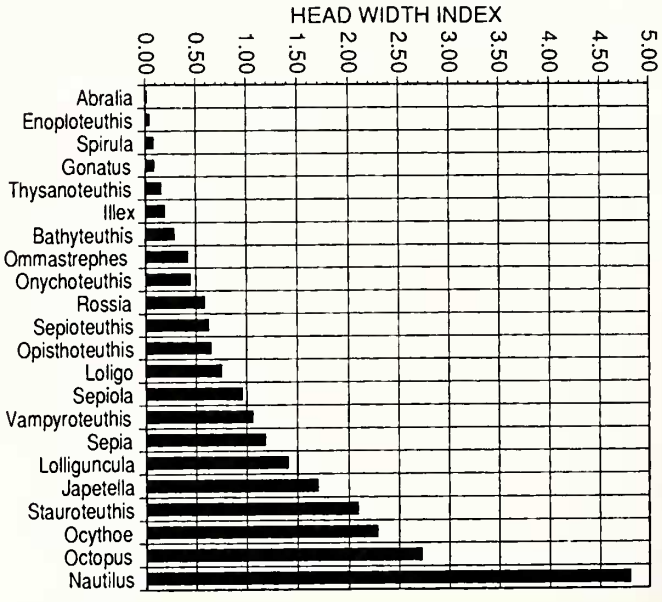

Fig 14. Histogram showing head-width index values for 22 taxa (Character 44).

gland in coleoids. As a result, we coded *Nautilus* as state 0. We had difficulty evaluating the character states in preserved animals and have concerns whether the states are adequately defined. While our attempt to determine and survey states is presented in the data matrix, we excluded this character from the analysis.

Character No. 46: Digestive gland. Character states: 0 - multiple digestive glands; 1 - two separate digestive glands (unipaired); 2 - digestive glands fused (single gland).
Comments. In coleoids the digestive gland consists of either two separate lobes or a single lobe. Separate but adjacent digestive glands can appear to be a single organ but are separated by a membrane and, in fresh specimens, can be pulled apart without damage to either organ. The definition of "separate" requires the lobes to be separate over their entire length. Within the coleoids, the appearance of two anlagen of the digestive gland in the embryos of *Loligo* and the presence of two digestive gland ducts in all coleoids indicate that unipaired glands (state 2) is the plesiomorphic state.

Character No. 47: Longitudinal muscle on mantle. Character states: 0 - present; 1 - absent.
Comments. We systematically sectioned the mantle in the region of the mantle-locking apparatus for the presence of a layer of longitudinal muscles. Generally a thin layer of longitudinal muscles was present external to the circular muscles on the outer surface. The longitudinal layer, however, is often discontinuous at least in the region surveyed and in a few families was absent.

Character No. 48: Position of vena cava relative to intestine. Character states: 0 - intestine passes ventral to vena cava; 1 - intestine passes dorsal or anterior to vena cava.
Comments. The cephalic vein extends posteriorly from the head along or near the ventral surface of the visceral mass until it splits to form the two branches of the vena cava. The intestine, arising from the visceral nucleus passes dorsal or anterior to this bifurcation (Figs. 8E, F) or, in some cases, passes posterior then ventral to the bifurcation and, as a result, traps the vena cava within the U-shape of the digestive tract (Figs. 8A, B, 9). In preserved specimens the cephalic vein and vena cavae often have collapsed and cannot be followed. As a result, we generally used the position of the intestine relative to the position of the renal appendages as an indicator of the character. In *Nautilus* the terminal portion of the intestine lies on the ventral mantle wall rather than on the visceral mass and is thereby separated from the vena cava (Griffin, 1900). As a result, this character is not applicable to *Nautilus*.

Character No. 49: Posterior salivary gland. Character states: 0 - absent; 1 - posterior to brain; 2 - on or in buccal mass.
Comments. The posterior salivary glands generally lie posterior to the cephalic cartilage. However, Aldred *et al.* (1983) found that in *Cirrothauma* a single gland is present and it lies within the buccal mass. Ebersbach (1915) reported a similar situation for *Grimpoteuthis umbellata* (Fischer, 1883) (his *Cirroteuthis umbellata*, see systematic comments in Voss, 1988). This character was surveyed as a possible synapomorphy for the cirrates. *Nautilus* lacks posterior salivary glands.

Character No. 50: Position of gonad relative to the VP coelom. Character states: 0 - gonad mostly within the coelom; 1 - less than 50% within.
Comments. In most cephalopods, the gonad, except for its attachment sites, lies mostly within the VP coelom, that is, it is covered by the coelomic lining (Figs. 1A, D). In octopods, however, the gonad lies mostly outside the coelom in a gelatinous milieu (Figs. 1B, C, E). Unfortunately, we had difficulty in incirrate octopods in determining how much of the gonad was covered by coelomic lining although it appeared to be greater than 50%.

In animals as complex as cephalopods numerous additional characters that have potential phylogenetic value at this level remain to be identified and surveyed. We mention four that we were unable to survey.

(1) Funnel-locking apparatus (Fig. 10A). In many cephalopods a specialized locking apparatus locks the funnel to the inner surface of the mantle. The locking apparatus occurs in decapods and some octopods and is probably convergent in these two groups. We suspect that in decapods the funnel component has a cartilaginous base that is lacking in octopods. However, even if this proves to be a valid distinction, it cannot be polarized by the condition in *Nautilus*. We originally attempted to apply this definition to the mantle component of the locking apparatus but found a large variety of structures with cartilage only rarely being present.

(2) Suprabrachial commissure (Fig. 5D). Most octopods have a strong commissure that loops dorsal to the esophagus and connects the lateral regions of the left and right brachial lobes of the subesophageal region of the brain (Aldred *et al.*, 1983). In cirrate octopods this commissure lies beneath the posterior buccal lobe but is separated from it by connective tissue. A corresponding commissure exists in *Vampyroteuthis* but is small and lies just beneath the posterior buccal lobe (pers. obs.). A counterpart is unknown in the decapods. Surprisingly this commissure appears to be lacking in *Japetella* (J. Z. Young, 1977). Unfortunately this is another character that cannot be polar-

Table 2. Data matrix for 50 characters and 17 taxa. Asterisk indicates character used in this study; slash indicates two character states present.

	1-5					6-10					11-15					16-20					21-25				
				*		*	*	*	*	*							*	*		*	*				*
Bathyteuthidae	0	0	1	0	2	0	1	2	0	1	2	1	1	1	1	0	0	0	1	0	0	1	1	1	1
Enoploteuthidae	0	0	1	0	2	0	1	2	0	1	1	1	0	1	0	0	0	0	1	0	0	0	1	1	1
Gonatidae	0	0	1	0	2	0	1	2	0	1	2	2	0	1	0	0	0	0	1	0	0	1	1	1	1
Loliginidae	0	0	1	0	2	0	1	2	0	1	1	1	0	1	0	0	0	0	1	0	0	1	1	1	1
Ommastrephidae	0	0	1	0	2	0	1	2	0	1	1	1	0	1	0	0	0	0	1	0	0	1	1	1	1
Onychoteuthidae	0	0	1	0	2	0	1	2	0	1	1	1	1	1	0	0	0	0	1	0	0	1	1	1	1
Sepiidae	1	0	0	0	2	0	1	2	0	1	2	2	0	1	1	0	0	0	1	0	0	1	1	0	1
Sepiolidae	0	0/1	1/3	0/4	2	0	1	2	0	1	1/2	1/2	1	0	0/1	0	0	0	1	0	0	1	1	0	1
Spirulidae	1	1	0	2	2	0	1	2	0	1	2	2	1	1	1	0	0	0	1	0	0	1	1	0	3
Thysanoteuthidae	0	0	1	0	2	0	1	2	0	1	1	1	0	1	0	0	0	0	1	0	0	1	1	1	1
Bolitaenidae	0	1	3	4	0	2	0	0	2	0	0	0	1	0	1	1	2	1	0	1	1	0	0	1	2
Octopodidae	0	1	2	3	0	2	0	0	2	0	0/1	0/1	1	0	1	1	2	1	0/1	1	1	0	0	1	2
Ocythoidae	0	1	3	4	0	2	0	0	2	0	1	1	1	0	0	0	2	1	0	1	1	0	0	1	2
Stauroteuthidae	0	1	2	1	0	2	0	0	2	0	0	0	0	0	1	1	1	1	0	1	1	0	0/1	2	2
Opisthoteuthidae	0	1	2	1	0	2	0	0	2	0	0	0	0	0	1	1	1	1	0	1	1	0	0	2	1/2
Vampyroteuthidae	1	0	1	0	0	1	0	1	1	0	0	0	0	1	1	1	1	1	0	0	0	0	0	1	0
Nautilidae/Ancestor	1	?	0	?	1	0	0	?	?	?	?	?	?	?	?	?	?	0	0	0	0	1	0	0	?

	26-30					31-35					36-40					41-45					46-50				
	*		*	*		*	*	*	*	*	*			*	*	*		*					*		
Bathyteuthidae	0	0	0	0	1	1	0	0	0	1	0	0	1	0	0	0	1	0	0	0	2	0	1	1	0
Enoploteuthidae	0	0	0	0	1	1	0	0	0	1	0	1	1	0	0	0	1	0	0	0	2	0	1	1	0
Gonatidae	0	0	0	0	1	1	0	0	0	1	0	1	1	?	0	0	?	0	0	0	2	1	1	1	0
Loliginidae	0	0	0	1	0	1	0	0	0	1	0	1	1	0	0	0	1	0	1/2	0	2	0	0	1	0
Ommastrephidae	0	0	0	0	1	1	0	0	0	1	0	1	1	0	0	0	1	0	0	0/1	2	1	1	1	0
Onychoteuthidae	0	0	0	0	1	1	0	0	0	1	0	0	1	0	0	0	1	0	0	1	2	0	1	1	0
Sepiidae	0	0	0	1	0	1	0	0	0	1	0	1	?	0	0	0	1	0	2	0	1	0	0	1	0
Sepiolidae	1	0	0/1	1	0	1	0	0	0	1	0	0	1	0	0	0	1	0	1	0	2	0	0	1	0
Spirulidae	0	0	0	0	0	1	0	0	0	1	0	1	1	0	0	0	1	0	0	0	1	0	0	1	0
Thysanoteuthidae	0	0	0	0	1	1	0	?	?	1	0	1	1	0	?	0	1	0	0	0	2	1	1	1	0
Bolitaenidae	1	1	1	2	1	0	1	2	2	0	1	0	0	?	2	0	0	1	3	2	2	0	1	1	0
Octopodidae	1	1	1	2	1	0	1	2	2	0	1	0	?	0	2	0	0	1	5	2	2	0	1	1	0
Ocythoidae	1	1	1	2	1	0	1	2	2	0	1	0	0	0	2	0	0	1	4	1	2	0	1	1	0
Stauroteuthidae	1	1	1	0	0	0	1	2	2	0	0	0	0	1	2	1	0	1	4	0	2	0	1	2	1
Opisthoteuthidae	1	1	1	0	0	0	1	?	2	0	0	0	0	1	2	1	0	1	?	2	2	?	1	0	1
Vampyroteuthidae	2	0	0	0	1	0	0	1	1	1	0	0	0	0	1	0	0	1	2	0	2	0	0	1	0
Nautilidae/Ancestor	?	0	?	?	1	2	0	?	?	1	?	0	?	2	0	0	?	?	9	0	0	0	?	0	0

ized by *Nautilus*.

(3) Genital pocket. The genital pocket is an invagination of the integument lining the mantle cavity that surrounds the accessory spermatophore organs. Absence of a genital pocket in male cirrates could be a synapomorphy for this group. This is one of a probable suite of characters associated with the degeneration of spermatophore structure.

(4) Dorsal lobe of the nephridial sacs (Figs. 8A-D). These lobes (see Character 19) could prove to be a synapomorphy of the Vampyromorpha and Octopoda but are difficult to survey in preserved material.

ANALYSIS

We surveyed 50 characters in 17 families and these data are presented in Table 2. We eliminated four of these characters (Nos. 13, 38, 44, 45) from the analysis because of potential errors related to questionable definition of character states or accuracy of surveying the states. We also eliminated seven characters (Nos. 1, 5, 22, 23, 27, 30, 47) after determining that the plesiomorphic state was "present" and the apomorphic state was "absent." For these characters we could not determine if losses represented homology or involved homoplasy. Polarity was determined for these by outgroup comparison on MacClade. Some other characters fell so obviously into this pattern (*e. g.* presence/absence of fins) that they were not surveyed. Characters with "fused" as the apomorphic state also presented a problem in distinguishing between homology and homoplasy. Three characters (Nos. 19, 42, 46) were eliminated on this basis as their plesiomorphic condition was the unfused state (polarity for Character 19 based on outgroup condition and for 46 on ontogeny). We further eliminated

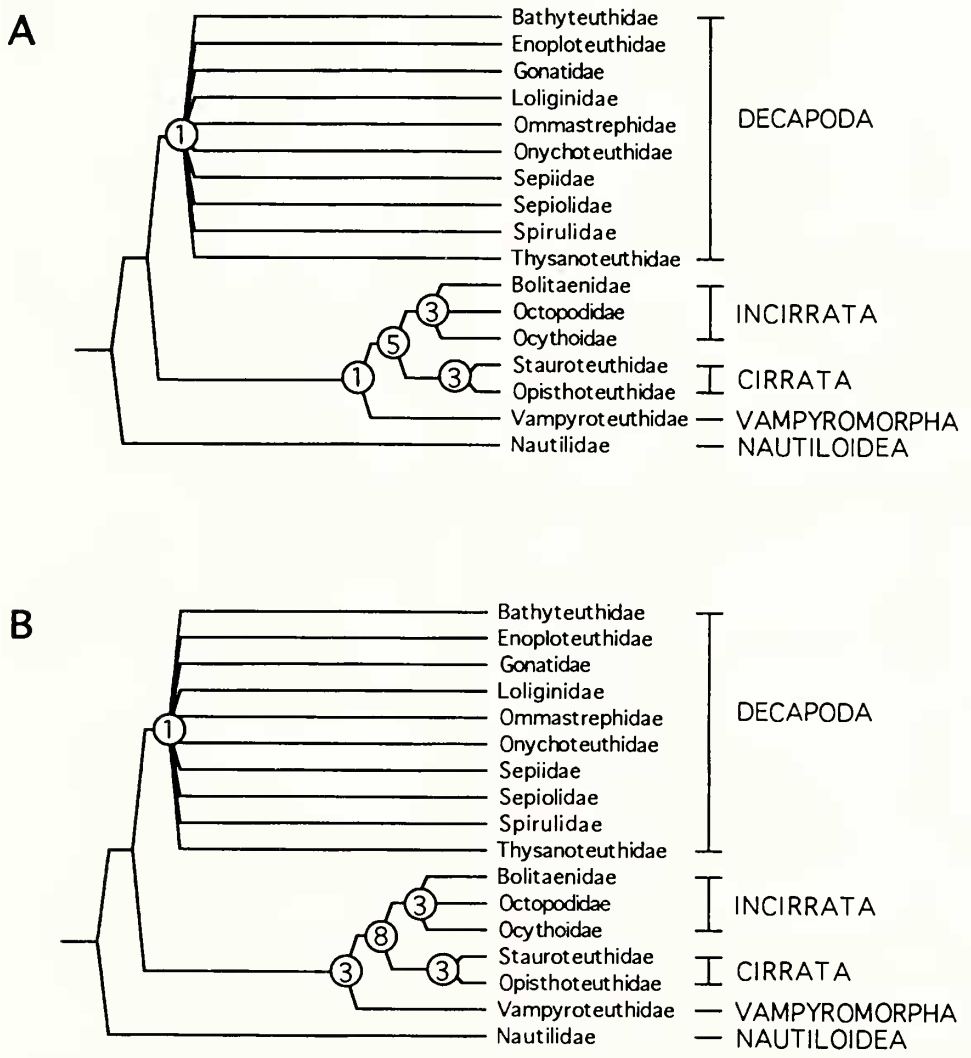

Fig. 15. Strict consensus of shortest trees from PAUP search of data set with 25 characters (14 trees; tree length 46). Numbers at internal nodes represent Support Indices. Indices of shortest trees: consistency index 0.98; homoplasy index 0.09; retention index 0.99; rescaled consistency index 0.97. Mickevich's consensus information from the consensus tree = 0.312. A. All character states unordered (Analysis I). B. Three characters with states ordered, others unordered (Analysis IIA). Bootstrap values (1,000 replicates) for nodes are: decapod node 70, octopod/vampire node 98, octopod node 100, incirrate node 97, cirrate node 97.

three characters (Nos. 2, 3, 50) because they might not be completely independent from other characters (*i. e.* Nos. 4, 4, 20, respectively) due to some overlap in definitions. One character (No. 37) was eliminated as it varied only within a major group (Decapoda) and was, therefore, uninformative at the level of interest in this study. Seven characters (Nos. 11, 12, 14, 15, 16, 24, 48) exhibited homoplasy under all plausible phylogenetic rearrangement of the groups. The worst of these was Character 24 (branchial canal), a presence/absence character with "absence" in *Nautilus* presumably defining the plesiomorphic state. All rearrangements resulted in multiple homoplasy, varying from three independent derivations to three independent losses of branchial canals with the minimum homoplasy involving two convergences. Until these seven characters can be redefined to eliminate homoplasy, we eliminate them from analyses. This left 25 characters.

We first analyzed the data (Analysis I, PAUP, Fig. 15A) with all characters entered as "unordered." To determine the strength of the nodes we determined the support index (SI) for all internal nodes (Eernisse and Kluge, 1993). Decapod monophyly (SI = 1) was supported unambiguously by only a single character (No. 7, Arms IV). However, eight other characters that we were not able to polarize (Nos. 8, Sucker rings; 9, Sucker stalks; 10, Sucker symmetry; 17, Fin cartilage; 31, Oviducal gland symmetry;

33, Photosensitive vesicles; 34, Subfrontal lobes; 43, DGDA/nephridial coeloms) have states that are, presently, found exclusively in the decapods and in all the decapods. In this first analysis only a single unambiguous character (No. 18, Statocyst outer capsule) change supported the vampyromorph/octopod node (SI = 1). Four additional characters (Nos. 10, Sucker symmetry; 17, Fin cartilage; 31, Oviducal gland symmetry; 43, DGDA/nephridial coeloms) could support this clade depending on which states prove to be plesiomorphic within coleoids. Monophyly of the Octopoda (SI = 5) was supported by five characters (Nos. 20, Visceropericardial coelom; 21, Dorsal mantle cavity; 28, Nuchal cartilage; 32, Oviducal gland position; 35, Arm-mantle muscle) and, potentially seven more (Nos. 6, Arms II; 8, Sucker rings; 9, Sucker stalks; 25, Mantle septum; 33, Photosensitive vesicles; 34, Subfrontal lobe; 40, Superior buccal lobe) depending on how polarity is resolved. The Cirrata (SI = 3) were supported by unambiguous changes in three characters (Nos. 39, Spermatophores; 41, Horizontal arm septa; 49, Posterior salivary glands) and one potential unpolarized character (No. 4, Shell shape). The Incirrata (SI = 3) were supported by unambiguous changes in three characters (Nos. 17, Fin cartilage; 29, Cornea; 36, Arm III hectocotylus) although the first is a "non-applicable" state resulting from fin loss.

We next analyzed the data (Analysis IIA, PAUP, Fig. 15B) after ordering three characters (Nos. 6, Arms II; 34, Subfrontal lobe; 40, Superior buccal lobe) whose evidence warrants this restriction (see Comments under each character). We consider this to be our best estimate of the phylogeny at this time. Topology and tree length did not change from the previous analysis. However support for some clades improved. Support for the decapod clade was unchanged, while the SI for the vampyromorph/octopod node increased to 3 from the additional unambiguous changes in two characters (Nos. 6, 40). Character 34 did not contribute to this node due to its ambiguous polarization at the coleoid node. All three ordered characters supported additional unambiguous character changes for the Octopoda which increased the SI to 8. Support for the Cirrata and Incirrata were unchanged. In both analyses the low degree of homoplasy in the data set utilized apparently resulted in the SI being identical to the number of unambiguous character changes at each node. We ran this same data set in Hennig86 (Analysis IIB) which provided the same topology for the six internal nodes (14 trees, tree length = 46, consistency index = 0.97, retention index = 0.99).

DISCUSSION

Analysis II provided marginal support for monophyly of the decapod clade (one character). However, the large pool of characters with states diagnostic of decapods but unpolarized suggests that additional support is likely. The vampyromorph/octopod clade seems reasonably well supported by three characters although some of these carry assumptions. Four unpolarized characters offer the possibility of additional support. The monophyly of the Octopoda was very well supported by eight characters with possible additional support from another three unpolarized characters.

Although monophyly of decapods, cirrate and incirrate octopods (Vampyromorpha is monotypic) was supported, we emphasize that not all families were included in our examination of these higher taxa. While we expect these higher taxa were adequately representated, confirmation from all families is needed.

The tree resulting from Analysis II rests on only 16 characters involving 18 character state changes. Ten characters defined the decapod, octopod, and octopod + vampyromorph clades. An additional six characters defined the two octopod clades. The remaining nine characters were effectively neutral (*i. e.* they neither added to nor substracted from support for the internal nodes). One of our primary obstacles in this study has been our inability to polarize many of the characters at the basal coleoid node. Because of this problem, eight of the nine neutral characters were "neutralized" and six of the other characters were only partially effective. As expected, PAUP analysis of the 16 characters gave the identical tree with identical SI values to that of Analysis II (tree length, of course, was shorter: 24) while analysis of the nine neutral characters resulted in a completely unresolved consensus tree.

In future analyses of decapod families with more nodes and a more complex hierarchy of nodes, emphasis must be placed on thoroughly understanding the interrelationships of characters that will enable determination of the basal clades upon which much of the polarization at more resolved nodes must rest. Efforts should, therefore, be placed on locating characters that have counterparts in outgroups (or can be polarized by ontogeny/paleontology), and that do not involve loss as the synapomorphy. Multistate characters in which evolutionary pathways can be reconstructed would be especially valuable. Homoplasy in these "basal" characters is dangerous but elimination of homoplasic characters is not only wasteful of any useful information they can contain but could be impossible, in a more complex and poorly understood phylogeny, by the method used here (*i. e.* recognition of which characters are homoplastic on the basis of "implausible relationships"). Only a thorough understanding of the relationships among character states through detailed study can guard against this pitfall. We simply argue in favor of an approach to phylogenetic studies by the methodology referred to by Mickevich (1995) as "synapomorphic cladistics" and long-recognized by many others (*e. g.* Bryant, 1989).

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Preliminary cladistic analyses of relationships among loliginid squids (Cephalopoda: Myopsida) based on morphological data

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Abstract: Most species in the cephalopod taxon Loliginidae have a near-shore habitat and are commercially important, yet phylogenetic relationships within the group have not been examined. In this study, relationships among loliginid species are analyzed using cladistic methods. Forty-eight morphological characters for 48 species (40 loliginid species and eight outgroup taxa) were collected through examination of museum specimens and primary literature and coded into a matrix for cladistic analysis. Both unweighted and successive weighting maximum-parsimony analyses were undertaken, and the phylogenetic signal of the data was evaluated. Unweighted analyses support the hypothesis of monophyly for Loliginidae, and suggest some well-supported sister species and crown-clade relationships (such as *Alloteuthis* Wülker and *Sepioteuthis* Blainville), but the positions of these groups relative to one another cannot be resolved due to the large number of most-parsimonious trees. Successive weighting analyses showed support for some additional major clades (*Photololigo* Natsukari and *Nipponololigo* Natsukari), and provided insight into the cladistic information value of the characters in the analysis. Continued collection of morphological and internal anatomical data for these species for all stages of the life cycle, as well as the addition of molecular data to the analysis, could help resolve relationships within the group.

The cephalopod taxon Loliginidae contains over 40 species of neritic squids found on most tropical and temperate continental margins around the world (Roper *et al.*, 1984). In many regions, these squids are found in high abundance near shore, particularly when spawning, and some species form integral links in coastal marine ecosystems (such as *Loligo opalescens* Berry, 1911, in Monterey Bay, California; see Morejohn *et al.*, 1978). Many loliginid species are commercially harvested (Roper *et al.*, 1984). In addition, the giant axons of certain loliginids (such as *L. pealei, L. vulgaris*, and *L. opalescens*) have served as important model systems in neurophysiological research (*e. g.* Young, 1938; Gilbert *et al.*, 1990; Rosenthal and Gilly, 1993).

Despite the ecological, economic, and scientific importance of loliginid squids, their phylogeny remains unresolved, and their taxonomy is confused (Voss, 1977). Numerous recent works (Natsukari, 1983, 1984; Brakoniecki, 1986; Alexeyev, 1992; Vecchione *et al.*, in press) have sought to clarify loliginid taxonomy using key morphological characters. Brakoniecki (1986), who examined loliginid hectocotylus morphology, grouped the species he studied into six hectocotylus types. He based a new generic-level classification on his findings, and proposed an evolutionary zoogeographic scenario for the radiation of the group. Vecchione *et al.* (in press) have used a phenetic analysis of loliginids as the basis of a generic-level taxonomy. Others, including Augustyn and Grant (1988) and Brierley and Thorpe (1994), have used allozyme electrophoresis to address problems of loliginid relationships. Despite such efforts, these authors admit that further work is necessary to clarify loliginid phylogeny.

No researchers have explicitly addressed loliginid phylogenetic relationships using cladistic methodology. Cladistic analysis allows many discrete character data to be considered simultaneously using an explicit, simple optimality criterion in which the preferred tree is the one which requires minimal assumptions of convergence and reversal across all characters in the analysis (the criterion of maximum parsimony) (Edwards and Cavalli-Sforza, 1964; Camin and Sokal, 1965; Kluge and Farris, 1969; Fitch, 1971; Farris, 1983; Sober, 1988; see Wiley *et al.*, 1991, for a review of cladistic philosophy and techniques). Cladistic analysis yields a hypothesis of phylogenetic relationships with which to interpret biogeographical patterns and character evolution (Brooks and McClennan, 1991; Maddison and Maddison, 1992). Phylogenetic hypotheses can also be used to construct taxonomic schemes (de Queiroz and Gauthier, 1990, 1992).

For this study, many aspects of loliginid morphology (particularly the hectocotylus, arm and tentacle-club sucker rings, spermatophores, fins, and some aspects of

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internal anatomy) were coded into a matrix for cladistic analysis to examine species-level relationships within the family. These data were analyzed using a maximum-parsimony algorithm program, and the results were compared with traditional taxonomic schemes and recent reclassifications. The strengths and problems of using morphological characteristics for examining loliginid evolution are addressed, and topics for future research are briefly outlined.

MATERIALS AND METHODS

DATA COLLECTION

Forty-eight morphological characters for 40 species of loliginid squids and eight outgroup taxa were coded into a data matrix in MacClade 3.04 (Maddison and Maddison, 1992) (Appendix I). Character states were determined through direct study of museum specimens at the National Museum of Natural History, the California Academy of Sciences, and the Invertebrate Museum at the University of Miami Rosenstiel School of Marine and Atmospheric Science, as well as published species descriptions (Appendix III). Some described loliginid species [including *Loligo arabica* (Ehrenberg, 1831) and newly described species such as *Photololigo robsoni* (Alexeyev, 1992)] were not included in this study because descriptions of these species were insufficient to code many characters with confidence, and specimens were not available for examination at the institutions I visited.

The outgroup method (Watrous and Wheeler, 1981; Maddison *et al.*, 1984) was used to polarize character transformations in this study. The eight outgroup taxa represent a broad range of decapod cephalopod diversity. Four oegopsid taxa (*Moroteuthis* Verrill, *Todarodes* Steenstrup, *Ctenopteryx* Appellöf, and *Bathyteuthis* Hoyle), two sepiolid taxa (*Euprymna* Steenstrup and *Rossia* Owen), and one sepiid taxon (*Sepia* Linné) were included, along with *Pickfordiateuthis pulchella* Voss, 1953. *Pickfordiateuthis* was originally described by Voss (1953) as a monospecific taxon closely related to Loliginidae as another member of the Myopsida. Recently, two new species of *Pickfordiateuthis* have been described, and *Pickfordiateuthis* has been subsumed within Loliginidae (Brakoniecki, 1996). For this analysis, *P. pulchella* was used as a representative of this group of squids. These outgroups were selected for a variety of reasons. In some cases, earlier authors have suggested that certain oegopsid taxa are close relatives of Loliginidae (*e. g. Ctenopteryx*; Young, 1991). In contrast, Berthold and Engeser (1987) suggested that Loliginidae, Sepiolidae, and Sepiidae are all closely related members of the Myopsida. Morphological similarities also exist between loliginids and various active nektonic oegopsids like *Todarodes*. Due to this uncertainty, a diversity of cephalopod taxa were included as outgroups in this study.

Many characters included in this analysis (such as hectocotylus morphology, sucker ring dentition, and fin shape) have been used in traditional studies of loliginid taxonomy, but have never been objectively analyzed simultaneously. In certain cases, some of these characters have been presumed to be informative at some taxonomic levels but not at others. For example, arm-sucker ring dentition generally has been used to distinguish between very similar species (Natsukari, 1983; Brakoniecki, 1986), but it has not been used as a taxonomic character above this level. In other cases, some characters have been examined only in those supraspecific taxa where they help unite or separate species (*e. g.* number of trabeculae per marginal club sucker in *Alloteuthis* Wülker; Hanlon *et al.*, 1992), and might not have been thoroughly examined in all loliginid species. Still other characters (*e. g.* spermatophore morphology) have been examined widely in loliginids, but have not figured importantly so far in cephalopod systematic studies (Hess, 1987; deMaintenon, 1990). Some characters were found to vary among loliginid species but were consistent within species, and were included in this analysis. Certain characters of traditional importance were avoided because they appeared to vary within certain species, and too few specimens were available to resolve these inconsistencies. For example, thickenings of the lateral edges of the vane of the gladius are for some authors important diagnostic characters for the genera *Doryteuthis* Naef and *Loligo* Lamarck but have been found to be variable within species (Cohen, 1976; Toll, 1982). Despite this, some polymorphic characters (characters that vary within some terminal taxa) were included in the analysis. Characters exhibiting intraspecific variation can contain strong phylogenetic signal (although generally not as strong as fixed characters) and thus should not be ignored or simply coded as fixed in cladistic analyses (Wiens, 1995). *A priori* assumptions about the information value of characters (other than inclusion of "traditional" well-studied characters in the analysis) were avoided, but inevitably (as in all phylogenetic studies) some characters that could be phylogenetically informative have been excluded. Appendix II lists the characters, argumentation and coding scheme used in this analysis.

Data for some characters are either not applicable for certain taxa ("n" in Appendix I) or could not be determined ("?" in Appendix I). Inapplicable characters usually refer to some aspect of a structure which is not present in all taxa in the analysis (*e. g.* "hectocotylus dorsal row sucker morphology" in taxa which do not possess hectocotyluses). In some cases, coding of "inapplicable" characters in this manner can cause problems in cladistic analyses (Maddison, 1993), but the solution advocated by Maddison

(1993) – combining all such characters into one character with many states – is often impractical and can lead to the loss of phylogenetic information. For example, in the case of this analysis, fusing all nine hectocotylus characters into one multistate character with many states was not performed. Fusing all hectocotylus characters into a single character with many distinct unordered states does not allow homology statements for individual aspects of hectocotylus morphology. It is possible, for instance, that species that possess a particular type of modified sucker, irrespective of the region of the arm that bears the modification, constitute a monophyletic group relative to species with other types of sucker modification, or vice versa. If the hectocotylus characters were fused into one unordered multistate character, this information would be lost – only species with almost exactly the same hectocotylus morphology (*i. e.* the same coded state for the single hectocotylus character) would be grouped together. Maddison's (1993) method is valid, but maximally conservative, and a great deal of phylogenetic information could be lost by collapsing characters in this way.

DATA ANALYSES

These data were analyzed using the maximum parsimony program PAUP 3.1.1 (Swofford, 1993). When terminal taxa were coded as having multiple states for one or more characters, these characters were interpreted as "polymorphic." All characters were unordered binary or multistate characters, and were weighted equally for all initial analyses. The use of equal weighting does not mean that each character in the matrix is of equal informative value. I have chosen to use equal weighting simply because I have no compelling reason to use any particular *a priori* differential weighting scheme (see discussion in Eernisse *et al.*, 1992). Heuristic searches were performed with 100 replications of random stepwise addition of taxa using tree bisection-reconnection swapping with one tree held at each step. The maximum number of trees stored for each search (MAXTREES) was 10,000. The COLLAPSE option was turned off for some analyses in an effort to find all regions of "islands" of most-parsimonious trees (Maddison, 1991; Swofford, 1993). Following Maddison's (1991) suggestion, a total of ten heuristic analyses were done to search for other islands and to examine the level of support for various branches within cladograms. A strict consensus cladogram was computed from the trees from each of the ten heuristic searches, for a total of ten strict consensus cladograms. A strict consensus of these ten strict consensus trees (a "grand strict" consensus cladogram) collapsed all ambiguities and revealed elements common to all trees from all ten searches.

After these preliminary analyses, two methods were used in an attempt to reduce tree number and investigate the phylogenetic utility of individual characters. The "reweight characters" option in PAUP was used to successively weight characters after each heuristic search (following the approach of Farris, 1969). Farris (1989) proposed the rescaled consistency index (RCI) and suggested its use in successive weighting analyses. In this analysis, characters were reweighted on the basis of their best rescaled consistency index value across the trees from the previous search. A heuristic search (following the same parameters as described above) was performed using the reweighted characters. Rounds of successive weighting were repeated until overall strict consensus tree topology did not change from one round to the next, or until character weights did not change after reweighting. As in preliminary analyses, strict consensus cladograms from the final round of weighting were combined, and a grand strict consensus cladogram was computed. This allowed common elements found in all successive weighting analyses to be determined.

A recently described technique called "safe taxonomic reduction" (Wilkinson, 1995) was also used in an effort to reduce the number of trees found. Analysis of matrices containing taxa with many missing data can result in an inordinately large number of equally parsimonious trees, because taxa with a large percentage of missing data (termed negatively underdetermined taxa) can occupy a number of equally parsimonious positions (Wilkinson, 1995). Consensus methods can be used to find common elements across multiple most-parsimonious trees (MPT's), but negatively underdetermined taxa can obfuscate patterns of relationship among other taxa that are found in all trees, yielding an extreme lack of resolution in strict consensus cladograms. The goal of safe taxonomic reduction is to remove negatively underdetermined taxa from the analysis without losing information about relationships (*i. e.* without altering patterns of relationships among the remaining taxa). This reduction in the number of negatively underdetermined taxa often reduces greatly the number of MPT's found by parsimony analysis. Increased resolution in consensus cladograms is often found after safe taxonomic reduction. In these analyses, the only taxa that could be safely removed from the ingroup using Wilkinson's technique were *Alloteuthis africana* and *A. media*, which were taxonomic equivalents of *A. subulata*, and *Loliolus affinis*, which was a taxonomic equivalent of *L. hardwickei*.

The phylogenetic signal of the data was evaluated using the g_1 test of Hillis and Huelsenbeck (1992). Based on simulation data, Hillis and Huelsenbeck (1992) proposed the use of the g_1 statistic (a measure of skewness) as one way to evaluate the ratio of signal to random noise in phylogenetic data. They found that high degrees of left-skew in plots of random tree distributions or total tree distributions obtained through exhaustive searches correlated well with the success of parsimony methods in finding the true phy-

logeny in simulation studies. Ten thousand random trees were generated based on this matrix using PAUP 3.1.1 with multistate taxa interpreted as polymorphic. The g_1 from this random tree distribution was compared to 95% and 99% confidence-limit values obtained from simulations with 50 binary or multistate characters and 25 taxa performed by Hillis and Huelsenbeck (1992), which should provide an approximate conservative comparison. In addition, all clades or sister-species groupings found across all trees (*i.e.* groupings retained in the grand strict consensus) were constrained to examine if phylogenetic signal was clustered within these groups. If the g_1 value (a negative value in left-skewed distributions) increases greatly after these constraints are applied, a large proportion of the phylogenetic signal in the matrix can be clumped within the universally supported clades, and is not evenly distributed across all data (Hillis and Huelsenbeck, 1992).

RESULTS

All unconstrained, unweighted analyses of all characters and all taxa resulted in 10,000+ most-parsimonious trees — the MAXTREES limit of 10,000 was reached in all analyses. The strict consensus cladogram of all sets of 10,000 trees from all ten unconstrained and unweighted heuristic searches is shown in Fig. 1. Tree lengths and statistics of the constituent trees are shown in Table 1.

The strict consensus cladogram of the ten final strict consensus trees from successive weighting was marginally more resolved than the strict consensus of the unweighted analyses (Fig. 2). Characters that were maximally and minimally weighted after multiple rounds of successive weighting are shown in Table 2.

Employing safe taxonomic reduction and removing *Alloteuthis africana, A. media,* and *Loliolus affinis* from the analyses had no apparent effect on the number of trees found in either unweighted or successive weighting analyses, or in the topology of the strict consensus cladograms from these analyses. Ten thousand trees were still found in all analyses following the removal of these taxa. The relative positions of *A. subulata* and *L. hardwickei* alone were the same as their positions when all taxa were included in the analysis.

Table 1. Tree statistics for 10,000 most-parsimonious trees found in the ten unweighted heuristic analyses.

Indices for unweighted trees
Consistency index (CI) = 0.582
Homoplasy index (HI) = 0.572,
Retention index (RI) = 0.663
Rescaled consistency index (RCI) = 0.386
Treelength (TL) = 194

Table 2. Maximally and minimally weighted characters across all weighted analyses (based on best rescaled consistency index fit). Characters that vary within the ingroup are denoted by an asterisk (*).

Maximally weighted characters	
Character Number	Description
1	rachidian cusps of radula
2	lateral cusps of radula
5	number of arm sucker rows
14	retractile tentacles
15	trabeculae number*
20	photophores on ink sac*
25	ventral row modification*
29	fused crest in ventral row of hectocotylus*
38	spermatophore placement*
40	spiral filament
43	pores on ink sac*
46	muscular septum
47	nuchal cartilage
48	digestive gland
Minimally weighted characters (weighted to zero)	
Character Number	Description
4	arm sucker rings
10	central manus sucker teeth*
13	marginal manus sucker teeth*
16	buccal membrane lobes
18	buccal lappet sucker teeth
19	buccal membrane formula
27	length of hectocotylus*

The g_1 skewness test of Hillis and Huelsenbeck (1992) suggests the presence of significant phylogenetic signal in the unweighted data matrix. The g_1 value for 100,000 random trees derived from this data matrix was -1.275647. This value was appreciably below the critical values for 50 binary or 50 four-state characters for 25 taxa [95% confidence limit for binary characters = -0.10, for four-state characters = -0.12; 99% confidence limit for binary characters = -0.11, for four-state characters = -0.13, based on simulation studies (Hillis and Huelsenbeck, 1992)]. When this procedure was repeated with the universally supported clades retained (using the grand strict consensus as a constraint), the g_1 value for a distribution of 100,000 random trees was -0.502625. This value suggests that even when universally supported clades are constrained, significant phylogenetic signal remains.

DISCUSSION

A small number of ingroup sister-species and subclade relationships were supported in the multiple

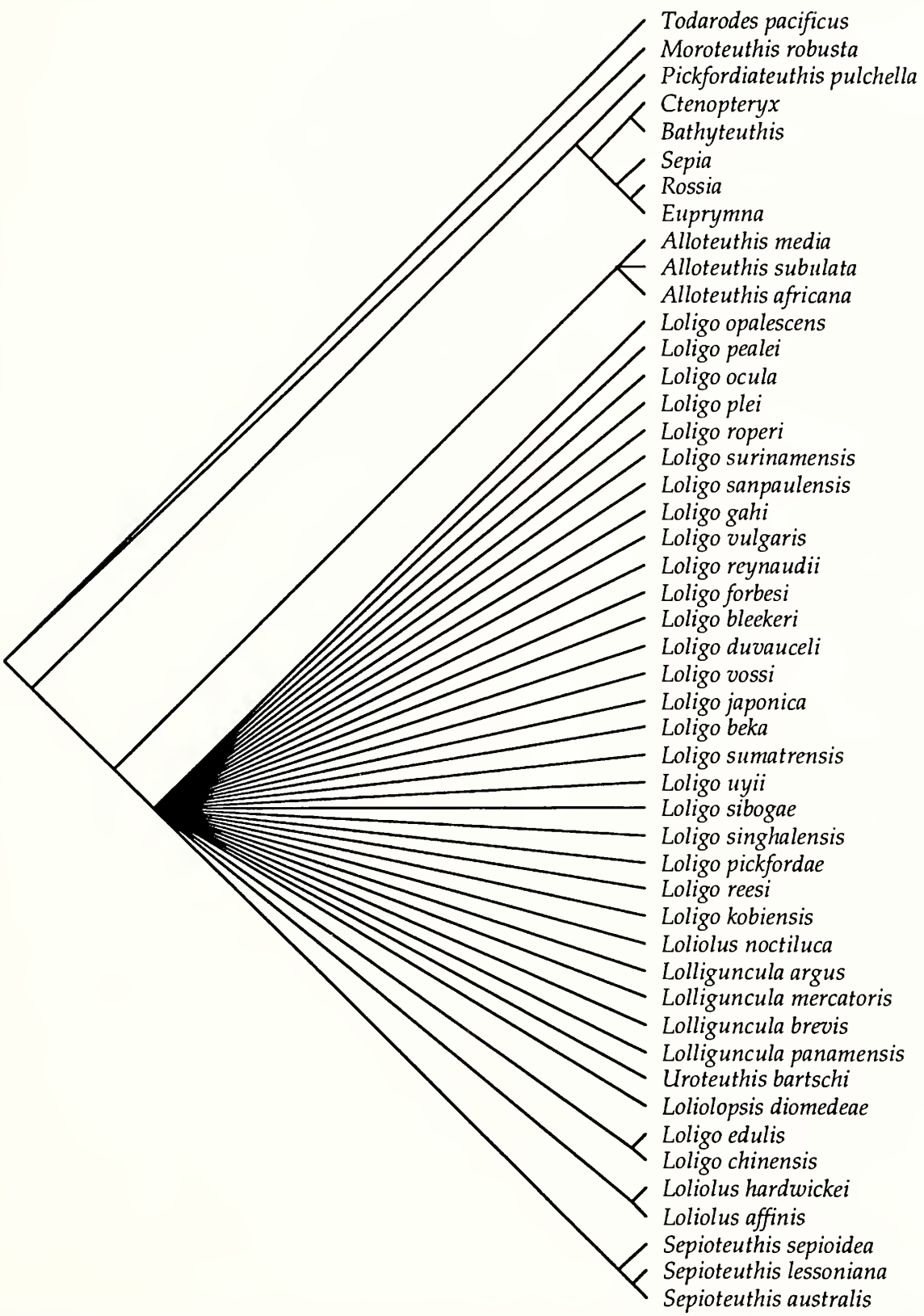

Fig. 1. Grand strict consensus cladogram of ten strict consensus cladograms derived from ten heuristic searches yielding 10,000 trees each. Tree statistics for the trees upon which this strict consensus is based are shown in Table 1.

unweighted heuristic analyses (Fig. 1), but deeper branching orders and relationships among loliginid squids remain unresolved, despite the relatively high phylogenetic signal suggested by the g_1 test. Successive weighting analyses consistently suggested support for some higher-level relationships (Fig. 2), but even with successive weighting and safe taxonomic reduction, tens of thousands of most-parsimonious trees were found, and, despite some interesting findings, the resulting grand strict consensus cladograms are largely unresolved.

Monophyly of Loliginidae was supported by all unweighted and weighted analyses. Myopsida (traditionally comprised of Loliginidae + Pickfordiateuthidae) was found to be paraphyletic in all unweighted and weighted analyses. Brakoniecki's (1996) inclusion of *Pickfordiateuthis* within Loliginidae is not justified based on these analyses. Within the ingroup, only four clades were consistently supported in all unweighted analyses, and two of these are sister-species groupings. Monophyly of the genus *Alloteuthis*, consisting of three species of small, slender squids found in the eastern Atlantic along the coasts of Europe and Africa (*A. africana, A. media,* and *A. subulata*) was supported in all analyses, and was the sister taxon to the rest of Loliginidae. *Alloteuthis* appears to have diverged from the other loliginid species early in the history of the group. Monophyly of the genus *Sepioteuthis* Blainville, which is comprised of *S. australis* and *S. lessoniana* (both Indo-West Pacific species), and *S. sepioidea* (a Caribbean species) was also supported in all unweighted analyses. Two synapomorphies appear to unite the three species of *Sepioteuthis* – a longitudinally oval fin shape uniquely derived within Loliginidae, and the large size of the dorsal row of papillae relative to the ventral row in the modified portion of the hectocotylus. In addition to these three-taxon clades, two sister-species pairings were consistently found – *Loligo chinensis* and *L. edulis* in one pairing, *Loliolus hardwickei* and *L. affinis* in the other. All other relationships within Loliginidae are unresolved in the unweighted analyses.

There are a number of possible explanations for this lack of resolution. First, the number of characters employed (48) is relatively low compared to the number of terminal taxa (48) included in the analysis. Another important factor might be that many of the characters used in the analysis are highly homoplastic, showing evidence of multiple convergences or reversals throughout the evolution of this group. Several of the characters included in this analysis have been used extensively in decapod cephalopod taxonomic studies, and appear to be very useful for distinguishing species, but when relationships among all loliginid species are studied and characters are atomized for cladistic analysis, individual characters can exhibit high levels of homoplasy.

Successive weighting techniques have been used by many authors to reduce the number of most-parsimonious trees, to increase resolution in consensus trees, as a heuristic tool to investigate the cladistic informativeness of characters, and to study the effect of homoplastic characters in cladistic analyses (Farris, 1969; Carpenter, 1988, 1994), but some authors have criticized the use of successive weighting techniques (Swofford and Olsen, 1990) or have urged caution in the interpretation of results from successive weighting analyses (Maddison and Maddison, 1992; Swofford, 1993; Suter, 1994). Some investigators (*e. g.* Suter, 1994) have found that the parameters used in successive weighting analyses can have an effect on the outcome of successive weighting analyses. In addition, successive weighting does not necessarily reduce the number of most-parsimonious trees – in this analysis, the tree buffer limit was reached on all analyses, unweighted or weighted. Successive weighting can reduce the weight of highly homoplastic characters to zero, effectively removing them from the analysis [seven characters were weighted to zero by the final round of weighting in these analyses (Table 2)]. As the number of characters actually included in the analysis drops, resolution in strict consensus cladograms likely will drop, particularly when the number of characters is small relative to the number of taxa in the analysis.

Farris (1969) and Carpenter (1988, 1994) have strongly supported successive weighting as simply an extension of the concept of cladistic reliability, or the degree of fit between a character and the phylogeny (Farris, 1969). Successive weighting allows *a posteriori* weighting based on the cladistic information value of the characters in the matrix. Characters that show little homoplasy when evaluated in conjunction with all other characters in the matrix are increased in weight (or set at a maximum base weight in PAUP 3.1.1) relative to characters exhibiting more homoplasy in subsequent rounds of analysis, while highly homoplastic characters are reduced in relative weight. Carpenter (1988, 1994) has argued that successive weighting "allows the characters of a given data set to judge themselves in terms of their reliability; that is, best fit to the solution supported by all the characters" (Carpenter, 1994: 216).

Successive weighting analyses supported all clades found in earlier, unweighted analyses, and suggested three other groupings not found in unweighted analyses. Two major clades were supported in all final weighted analyses – a clade consisting of all loliginid species possessing paired bioluminescent organs on the ink sac [*Loligo edulis, L. chinensis, L. duvauceli, L. sibogae, L. singhalensis, L. pickfordae* (Adam, 1954), *L. reesi, Loliolus noctiluca,* and *Uroteuthis bartschi*, all found in the Indo-West Pacific], and a clade consisting of seven other Indo-West Pacific species (*Loligo beka, L. japonica, L. sumatrensis, L. uyii, L. kobiensis, Loliolus hardwickei,* and *L. affinis*). In addition,

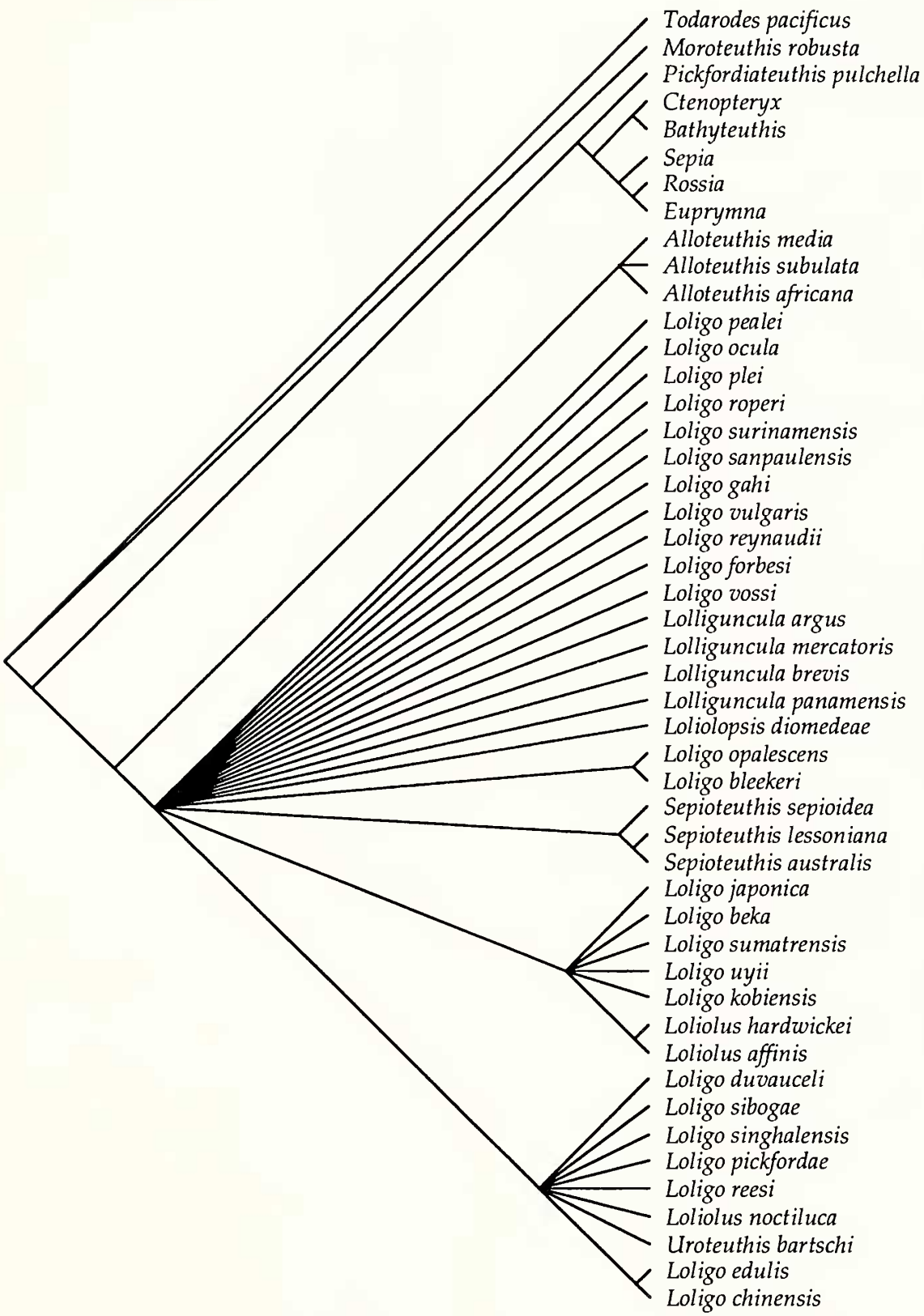

Fig. 2. Grand strict consensus cladogram of ten strict consensus cladograms from successive weighting analyses from each of ten heuristic searches.

Loligo opalescens and *L. bleekeri* were found to be sister species in all final weighted analyses.

The major putative synapomorphy uniting the bioluminescent loliginids are the paired bean-shaped bacterial photophores (luminescent organs) on the ventral side of the ink sac. This clade is similar to the proposed genus *Photololigo* Natsukari (1984), which includes five of these species (*Loligo edulis, L. duvauceli, L. chinensis, L. singhalensis,* and *L. sibogae*) as well as *L. arabica*, which was not included in this analysis. Natsukari's *Photololigo*, however, does not include *L. reesi, L. pickfordae, Loliolus noctiluca,* or *Uroteuthis bartschi*. Results of this weighted analysis support Vecchione *et al.*'s (in press) *Photololigo* as a monophyletic group. However, these authors divide *Photololigo* into two smaller groups – a subgenus *Photololigo* and a subgenus *Uroteuthis* (consisting only of one species – *U. bartschi*). It is possible that *U. bartschi* is a derived member of the photololiginid clade. If this is true, Vecchione *et al.*'s (in press) subgenus *Photololigo* is paraphyletic with respect to their subgenus *Uroteuthis*. Unfortunately, cladistic analysis of these data cannot address this question. Alexeyev (1992) has reported that some specimens of *Lolliguncula mercatoris* and a single specimen of *Loligo forbesi* appeared to possess photophores on the ink sac. In this analysis, these species are considered to lack photophores, pending further investigation (as suggested by Vecchione *et al.*, in press). If Alexeyev's (1992) findings are accurate, they must be accounted for in future phylogenetic studies of this group.

More detailed studies of the primary synapomorphy that unites the species of *Photololigo* – the photophores themselves – might help resolve these problems. In *Euprymna*, the bioluminescent organ is the product of a complex interaction between the squid and symbiotic luminescent bacteria (McFall and Ruby, 1991). Further investigations of this interaction and its effects on photophore morphology in all photololiginid squids (*e. g.* Haneda, 1963; Pringennies and Jørgensen, 1994) could illuminate species relationships within the clade.

The other major clade found in the weighted analyses is very similar to Natsukari's (1983) *Nipponololigo*, a proposed subgenus of *Loligo* comprised of *L. japonica, L. uyii, L. kobiensis,* and *L. beka*. The successive weighting analyses support the inclusion of *L. sumatrensis* and the *Loliolus affinis-hardwickei* sister-species grouping within a broader *Nipponololigo* clade. The two synapomorphies uniting these species are the sucker morphology of the dorsal and ventral rows of the hectocotylus. The pedicels of the dorsal row suckers are fused with their protective membrane and widened into fleshly slabs (Natsukari, 1983; Brakoniecki, 1986). In most of these species, the slabs retain small suckers; in *L. affinis* and *L. hardwickei*, however, the suckers are not present on the tops of the slabs. In these analyses, the lack of suckers on the tops of the slabs was revealed as a synapomorphy uniting these two species as sister taxa. In the ventral row of the hectocotylus, all species in the *Nipponololigo* clade possess minute, apparently suckerless papillae. Vecchione *et al.* (in press) have proposed the name *Loliolus* to include all members of Natsukari's *Nipponololigo* as well as *L. affinis* and *L. hardwickei*. These species are divided into two subgenera – *Loliolus (Loliolus)* (comprised of *L. affinis* and *L. hardwickei*) and *Loliolus (Nipponololigo)* (comprised of the species in Natsukari's *Nipponololigo*, plus *L. sumatrensis*). As with *Photololigo,* these analyses generally support their conclusion, although a paraphyletic *Nipponololigo* (with respect to *L. affinis* and *L. hardwickei*) is a possibility that cannot be addressed with these data alone.

Loligo opalescens and *L. bleekeri* constitute a sister-species pairing in all weighted analyses. Brakoniecki (1986) anticipated this result. He proposed that the epithet *Doryteuthis* (subgenus *Doryteuthis*) be applied to six species of loliginid squids. Five of these species (*Loligo plei, L. roperi, L. sanpaulensis, L. gahi,* and *L. opalescens*) are found in American waters, while one species (*L. bleekeri*) is found only in Japanese waters. *Doryteuthis (Doryteuthis)* and *Sepioteuthis* are the only geographically disjunct groupings described by Brakoniecki (1986). Brakoniecki proposed a causal explanation for the distribution of *Doryteuthis (Doryteuthis)* – he suggested that a slight rise in water temperature in the northern Pacific Ocean could have allowed the *L. bleekeri-opalescens* common ancestor to disperse from the eastern Pacific coast of North America to Japan via the Aleutians. The results of the weighted analyses support a sister-species relationship between *L. bleekeri* and *L. opalescens*, but, due to the lack of resolution of relationships among other *Doryteuthis (Doryteuthis)* species, a monophyletic subgenus *Doryteuthis* (*sensu* Brakoniecki, 1986) remains a possibility, but is not directly supported. Due to the overall lack of resolution, the possible ancestral range of the *L. bleekeri-opalescens* ancestor cannot be examined.

In addition to these putative clades, certain species presently grouped in the genus *Lolliguncula* (*L. panamensis, L. mercatoris,* and *L. brevis*), together with *Loliolopsis diomedeae*, were found in all strict consensus trees from all weighted analyses. However, the position of *Lolliguncula argus* was variable across these trees. In some consensus trees, *L. argus* was completely outside the clade comprised of the rest of the *Lolliguncula* species plus *Loliolopsis diomedeae*. In other trees, *L. argus* was found to be a highly derived member of the *Lolliguncula* + *Loliolopsis* clade. Due to the variable position of this taxon, the *Lolliguncula* + *Loliolopsis* clade collapsed in the overall strict consensus cladogram (Fig. 2).

Successive weighting analyses can provide heuristic

insight into the information value of the characters in the analysis. Most informative, consistent characters found after multiple rounds of successive weighting are invariant within the ingroup (Table 2). Few characters that vary within the ingroup appear to have high rescaled consistency indices across all initial unweighted trees. Also, several characters show varying amounts of homoplasy across initial unweighted most-parsimonious trees, and subsequently have low weights after successive weighting analyses.

The overall lack of resolution in strict consensus trees found after these unweighted and weighted analyses and the low weights of many characters after successive weighting highlight the limited utility of using only gross external morphological characters to investigate loliginid squid phylogeny. Despite this general conclusion, external morphological characters should not be ignored in future investigations of loliginid relationships. The Hillis and Huelsenbeck (1992) g_1 test shows significant phylogenetic structure in the data matrix. Some of the characters used in this analysis do appear to carry appreciable phylogenetic information. Undoubtedly, more data must be gathered to test the results of these analyses and to resolve relationships among these squids. Little is known about comparative internal anatomy in loliginid squids, although excellent studies have been done of particular species (*e.g.* Williams, 1909). For example, the anatomy of the nervous system and circulatory system, and perhaps aspects of juvenile development, are particularly promising systems for inclusion in cladistic analysis. Many neurophysiological studies have been performed on a broad range of loliginid squids, including *Loligo opalescens, L. pealei, L. vulgaris, Sepioteuthis lessoniana, Lolliguncula brevis,* and *Alloteuthis media* (*e.g.* Brown *et al.*, 1991; Chrachri and Williamson, 1993; Fishman and Metuzals, 1993; Preuss and Budelmann, 1995). Due to the ease of culturing some of these species in the laboratory (Lee *et al.*, 1994), and the giant axons of many loliginids, more comparative neurological studies undoubtedly will be performed. These data could be combined with other morphological and anatomical data for cladistic analysis.

In addition to internal anatomical information, molecular data could aid in resolving loliginid relationships. Recently, Yeatman and Benzie (1994) have found genetic evidence of cryptic speciation within *Photololigo edulis* and *P. chinensis*, providing evidence of the power of molecular techniques in species-level research of loliginid squids. An ongoing sequencing study of two mitochondrial genes (the 16S ribosomal DNA gene and the cytochrome *c* oxidase subunit I gene) could shed light on loliginid relationships (Anderson, unpub.). Relationships among loliginid squids at the species level and investigations of cladogenesis and biogeography within this group will be possible only through an examination of multiple sources of data, including morphological, anatomical, and molecular sequence data.

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APPENDIX 1 - Data Matrix. Inapplicable characters for particular taxa are indicated by "n", while missing or unknown character states are coded as "?". Certain taxa are polymorphic for particular characters. In these cases, the following code was used: "a" = states 0 and 1, "b" = states 1 and 2, "c" = states 0 and 2, and "d" = states 1 and 3. Taxon names above the species level are based on Nesis (1987).

	1111111111222222222233333333334444444444 1234567890123456789012345678901234567890123456789012345678
Todarodes pacificus	11?10102011010100n103nnnnnnnn2000????????2000010
Rossia	00?1a??0?110?1?20n001nnnnnnnn0111????????0011101
Moroteuthis robusta	11?00nn3?2???0??0n000nnnnnnnn2000?????2002?00010
Sepia	00?01nn200??01?01?002nnnnnnnn1nn10???????n011011
Ctenopteryx	???11??0nnnnn0??1?010nnnnnnnn3nn1?????20?0?00010
Bathyteuthis	00?11110nnnnn0?011100nnnnnnnn0110?????20?0?00010
Euprymna	00?0ann0nnnnn1?10?021nnnnnnnn01100??????0011101
Pickfordiateuthis pulchella	110a0?31?0???0?20?0021266?00?01110001?1000?11010
Loligo opalescens	11?10112011 0a0001100202 00?00?20010000a???0011010
Loligo pealei	11110112011110?0110021200?01?2001?000a0110011010
Loligo ocula	11?10112?11110?01?0021210?01?2001?0000???0?11010
Loligo plei	11110112011110?0110020110?01?2001100000110011010
Loligo roperi	11?10112011010?01?0020210?01?2001000000110011010
Loligo surinamensis	11?10112011110?01?0021210?00?2001?00000110?11010
Loligo sanpaulensis	11?10122011010001000 2001??01?2001?0000?100?11010
Loligo gahi	11?10112011010001000 2001??01?2001100000110011010
Loligo vulgaris	11?10112010 0?0?0110020111?00?2001100 0????011010
Loligo reynaudii	11?10a12?a1010?01?00201bb?00?2001?0000011??11010
Loligo forbesi	11?10132?110?0?0110020111?01?2001?0000?????11010
Loligo bleekeri	11?10???2?????0?01?0020100?00?2001100 0?????11010
Loligo duvauceli	11?10112111010001?03201111 0 0?2001?2200?1?0011010
Loligo edulis	11?10112111110001003201221 0 0?2001122 0 0?1??011010
Loligo chinensis	11?10102111110?0110320122000?2001011 0 0?1??011010
Loligo vossi	11?10012011010?01?0020111 10??2001 1????????11010
Loligo japonica	11?10112011010001?0020154?0012001?1100????011010
Loligo beka	11?101b2011010?01?0020154?0012001?1100?1???11010
Loligo sumatrensis	11?10112010010?01?0020154?0??2001 0000??1??011010
Loligo uyii	11?10122 0??10001?0020154?0012001?1100?????11010
Loligo sibogae	11?10112111 0?0001?03201221 0 0?2001 1???0?1??011010
Loligo singhalensis	11?101a2111110?01?03201111 0 0?2001?00001100011010
Loligo pickfordae	11?10012?110?0?01?03201??????20011????????011010
Loligo reesi	11?10112111010?01?0320111?10?2001 000?0110011010
Loligo kobiensis	11?1012200??10001?0020154?0012001?02100110011010
Alloteuthis media	11?1011201aa10100n00201bb100?212100010???1011010
Alloteuthis subulata	11?1011201aa10100n00201bb100?2121000100111011010
Alloteuthis africana	11110112011 0?0100n00201bb100?212100010???1011010
Loliolus hardwickei	11010122110010001100 2354?0002101022?02110111010
Loliolus affinis	11?10122110 0?0001?0022354?c0021010???0?110111010
Loliolus noctiluca	11?1011200??0000100322311?10?21010???01110011010
Sepioteuthis sepioidea	11010112110010000n0020111200?1nn100020???0011010
Sepioteuthis lessoniana	11?10002111010001100201112 00?1nn1000001110011010
Sepioteuthis australis	11?10002111010001100201112?0?1nn10000001 1?011010
Lolliguncula argus	11?10112011 0?0?00n00300d??a0?211101003010 0011010
Lolliguncula mercatoris	11?10022?110?0?00n00201333 01?211102202?????11010
Lolliguncula brevis	11?10122111010001?0020a3a?a0?2111000010100011010
Lolliguncula panamensis	11?10122111aa00011002003??10?2111000020100011010
Uroteuthis bartschi	11210112111 0?0001?03201010 00?2021100000111011010
Loliolopsis diomedeae	11110112011 0?0?01?0020135?10?211100001???0011010

APPENDIX II. Character Descriptions and Codings. Supplemental sources of information used are listed by each character (or each character suite) with the exception of particular references that pertain to certain taxa, including: *Bathyteuthis* (Roper, 1969); *Pickfordiateuthis pulchella* (see Voss, 1953; Brakoniecki, 1996); *Alloteuthis africana* (see Adam, 1950) *Loligo pealei, L. ocula, L. plei,* and *L. roperi* (see Cohen, 1976); *L. sanpaulensis* and *L. gahi* (see Brakoniecki, 1984); *L. chinensis* (see Natsukari and Okutani, 1975; Nateewathana, 1992); *L. edulis, L. beka, Loliolus affinis, Loligo sumatrensis* (see Nateewathana, 1992); *L. surinamensis* (see Voss, 1974); *L. kobiensis/Loliolus rhomboidalis* (see Burgess, 1967); *Loligo sibogae* (see Adam, 1954; Natsukari, 1976); *L. pickfordae, L. duvauceli, L. singhalensis* (see Adam, 1954); *Loliolus* (Lu *et al.,* 1985); *Sepioteuthis* (Lu and Tait, 1983); *Lolliguncula argus* (see Brakoniecki and Roper, 1985); *L. panamensis* (see Berry, 1911; Brakoniecki, 1980); *Uroteuthis bartschi* (see Adam, 1954; Rehder, 1945; Voss, 1963), and *Loliolopsis diomedeae* (see Berry, 1929). Characters are grouped by system; numbers refer to the position of the character in the data matrix (Appendix I).

A. Radula

1. Rachidian tooth (unicuspid/tricuspid) - Within most squids, the radula is comprised of seven longitudinal rows of teeth – a central rachidian, a pair of first and second lateral teeth, and a pair of marginal teeth. The rachidian tooth is usually either composed of a single cusp (unicuspid), or has a large central tooth with two lateral cusps (tricuspid).
2. Lateral teeth (unicuspid/bicuspid) - The first and second lateral teeth within loliginids are comprised of two cusps, while the first and second lateral teeth in numerous other squids are unicuspid.

B. Arm/tentacle club/sucker rings

3. Brachial cartilage (absent/fibrous type/hyaline type) (deMaintenon, 1990) - The brachial cartilage is a small cartilaginous structure found in many squids located antero-ventrally to the cranial cartilaginous body. The brachial cartilage seems to serve as a base for the tentacles and fourth arms. Some loliginids (*e.g. Sepioteuthis sepioidea*) lack this structure altogether, some have either a variable region of fibrous connective tissue (coded as "fibrous type"), and still others possess a distinct block of hyaline cartilage ("hyaline type").
4. Arm sucker rings (smooth/with teeth) - All taxa in this analysis possess horny chitinous rings in their arm suckers. These rings are either smooth, or they possess teeth of various shapes.
5. Arm sucker rows (two/four) - All loliginids and many other squids possess two rows of stalked suckers running along the arms. Many cephalopods possess four rows of suckers along the inner surface of the arms.
6. Arm sucker teeth position (all around ring/only on distal edge) - In most loliginids, the teeth on the chitinous sucker rings of the large proximal suckers on the third and fourth arms are found only on the distal edge of the sucker rings. In particular species, the sucker ring teeth are found all around the ring (although decreasing in size in the proximal region of the ring).
7. Arm sucker teeth shape (sharp/square or rounded and blunt/low, wide and flat/small, low and rounded) - A great diversity of arm sucker tooth shape can be found among loliginid species. Teeth are generally either tall, slender, and sharply pointed (as in *Loligo chinensis*), tall with rounded tips, or relatively flat and wide (often considerably wider than tall). Some (such as *L. japonica*) possess low rounded teeth, usually slightly wider than tall, with rounded, half-circle edges. The lone specimen of *L. forbesi* examined possessed a unique tooth morphology, consisting of very small, irregular teeth, giving the ring a pebbly appearance.
8. Club morphology (many tiny suckers/two rows in manus/four rows in manus/no marginal suckers or distinct dactylus) - The number of sucker rows in the manus region of the tentacle clubs is variable among squids. Many taxa (such as *Bathyteuthis*) possess a large number of minuscule suckers on the tentacle clubs, with no distinct regions. Other squids possess a distinct carpus, manus and dactylus, with two rows of suckers in the manus region. All loliginid squids possess a distinct manus and dactylus, with four rows of suckers (two central rows and two outer marginal rows) in the manus. *Pickfordiateuthis* possesses a few large, central suckers in the manus, with no marginal suckers and no distinct dactylus.
9. Central club sucker size (much larger than marginal suckers/similar in size to marginal suckers) - There is substantial variation in the size of the central club suckers relative to that of the marginal club suckers. In some loliginid species, marginal club suckers are nearly as large as central suckers while, in others, the marginal suckers are considerably smaller than the nearby central suckers.
10. Central manus sucker teeth (absent/present/hooks) - Some loliginid species possess smooth, toothless chitinous rings in their largest central club suckers. Most loliginids have teeth of some kind on their central manus sucker rings. Some outgroup taxa possess sharp hooks in their club suckers.
11. Central manus sucker teeth shape (blunt/pointed) - Central club sucker teeth are generally sharp and pointed, but some species have central manus suckers with teeth with rounded or blunt tips.
12. Central manus sucker teeth pattern (uniform sizes/many with alternating small and large teeth) - Patterns in central club sucker teeth sizes are variable, even among suckers on one tentacle club. In general, however, teeth are subequal in size on each individual ring. In some species, teeth show an alternating pattern (often large-small-large-small). Some species show more complex patterns of alternating small, medium and large teeth.
13. Marginal manus sucker teeth shape (blunt/pointed).
14. Retractile tentacles (absent/present).
15. Trabeculae number per marginal club sucker (one per marginal sucker/two per marginal sucker) - Most loliginid species possess thick trabeculae (muscular supports for the protective membranes of the tentacle clubs) spaced evenly between the marginal club suckers, averaging one trabecula per marginal sucker. Other species (members of the genus *Alloteuthis*) possess two trabeculae (Roper *et al.*, 1984) attached near the base of each marginal sucker.

C. Buccal lappets

16. Buccal lappet lobes (seven/eight/no lobes) - The number of buccal lappet lobes is variable among squids, and has been used as a taxonomic character. All loliginid squids possess seven buccal lappet lobes.
17. Buccal lappet suckers (absent/present) - Most loliginid species have tiny suckers on the inner surface of their buccal lappets. The three species of *Alloteuthis* and *Sepioteuthis sepioidea* do not have suckers on their buccal lappets.
18. Buccal lappet sucker teeth (absent/present).
19. Buccal membrane formula (DDVV/DDVD) - The location of the buccal lappet supports relative to the arms has commonly been used in cephalopod systematic studies. In loliginids and many other squid groups, the buccal lappet supports are attached to the

dorsal edges of the first and second arms, and to the ventral edges of the third and fourth arms (this pattern is often abbreviated "DDVV"). In other squids, the supports are attached to the dorsal edge of the first, second and fourth arms, and to the ventral edges of the third arms (abbreviated "DDVD").

D. Photophore morphology

20. Photophores on ink sac (absent/one round photophore/one U-shaped photophore/two bean-shaped photophores) - Photophores (bioluminescent organs) of various types are widespread throughout many cephalopod taxa. Most taxa examined in this study lack photophores. Some (*Ctenopteryx*) possess a single large round photophore on the ink sac. Others (*Euprymna*) possess a large U-shaped photophore on the ventral surface of the ink sac. Some loliginid species possess two oval or bean-shaped photophores. These species have been grouped in three separate genera (*Loligo, Uroteuthis,* and *Loliolus*) by earlier authors, while recent workers have suggested that loliginids with photophores constitute a natural group (named *Photololigo*). Because photophore shape and number varies across Loliginidae, *Ctenopteryx,* and *Euprymna*, these structures have been coded as different states. Because photophore number and basic external morphology is similar across all loliginid species with photophores, these structures have been coded as putative homologues for this analysis. Only through cladistic analysis of many characters can individual statements of homology such as this be assessed (the test of congruence; Patterson, 1982).

E. Hectocotylus morphology (Brakoniecki, 1986)

21. Hectocotylized arms (none/left dorsal arm/left ventral arm/right ventral arm) - The hectocotylus is a modified arm (or arms) in males that aids in transfer of spermatophores to the female. Hectocotyluses can exhibit radically different sucker morphology from the other arms, or can be of a very different length from the rest of the arms. Different cephalopod taxa have different arms hectocotylized, or lack an obvious hectocotylus altogether.
22. Modified region in dorsal row of hectocotylus (distal suckers modified to tip of arm/central suckers only modified/all suckers modified) - The region of modified suckers in the dorsal row of the hectocotylus is variable across loliginid species. In most species, only the distalmost suckers show any sort of modification, extending to the tip of the arm. A few species (*Loliolus*) show sucker modification along the entire length of the arm. Other species show minimal sucker differentiation on the hectocotylus which is restricted to a central region of the arm.
23. Modified region in ventral row of hectocotylus (distal suckers modified to tip of arm/central suckers only modified/all suckers modified) - See description for character 22.
24. Type of sucker morphology in dorsal row (small suckers with large pedicels/tiny suckers with long triangular pedicel/robust conical suckerless papillae/long thin suckerless papillae/tiny papillae/small suckers and stalks) - Sucker modifications are extremely variable in loliginid hectocotyluses, but seem to fit into a few distinct classes, which may be related to one another in complex ways. Some hectocotyluses possess small suckers at the tip of large, thick, columnar stalks (pedicels). Others show a similar modification - tiny suckers at the tip of pedicels which are distinctly wider at the base than at the tip, giving them a triangular shape. Some species possess thick, conical "papillae" that appear to lack suckers of any kind, but come to a point at their tips. Others possess a similar, but distinct, sucker modification in which long, rounded finger-like papillae are found. Some species possess only minute papillae in the dorsal row of the hectocotylus. Finally, a few species have suckers that are slightly smaller than normal, but are otherwise unmodified.
25. Type of sucker morphology in ventral row (small suckers with large pedicel/tiny suckers with long triangular pedicel/robust conical suckerless papillae/long thin suckerless papillae/fused crest/no suckers/suckers embedded in swelling) - Most sucker modifications found in the dorsal row of the hectocotylus are also found in the ventral row. There are a few differences. In many species, the ventral row of suckers is present as a row of tiny papillae, similar in morphology to the "finger-like" papillae described above, but much smaller. In some species, the pedicels of the ventral sucker row are fused with the ventral protective membrane, resulting in a series of thickened slabs (a fused crest) in the ventral row. One species (*Loliolopsis diomedeae*) completely lacks suckers of any kind in the ventral row. The ventral row of suckers in *Pickfordiateuthis* appears to be embedded in a swelling, an autapomorphy of this taxon.
26. Size of suckers on hectocotylus (suckers of both rows about the same size/ventral row suckers larger/dorsal row suckers larger/dorsal row suckers larger proximally, ventral row suckers larger distally) - In many cases where the sucker modifications in both rows are the same, consistent differences in sucker height can be seen between the rows. In some cases, the suckers in each row are approximately equal in size, tapering to the tip of the arm. Alternatively, the suckers in either the dorsal or ventral row can be larger than adjacent suckers in the other row. In a few species, dorsal row suckers appear to be larger proximally, but rapidly decrease in size down the length of the arm, while suckers in the ventral row either increase in size, or decrease much more slowly, resulting in the dorsal row of suckers being larger proximally, but the ventral rows of suckers being larger distally.
27. Length of hectocotylus (same length as fellow arm/longer than fellow arm/shorter than fellow arm) - In most cases, the length of the hectocotylized ventral arm is approximately the same as the length of the non-hectocotylized ventral arm. In some cases, however, the hectocotylized arm is distinctly longer or shorter than the other ventral arm.
28. Ridge between sucker rows in modified region of hectocotylus (absent/present) - A fleshy ridge is evident between the sucker rows in the modified portion of the hectocotylus in some loliginid species. This ridge is lacking in males of most loliginid species.
29. Fused crest in ventral row (without suckers/with suckers) - In squids with a fused crest ventral row modification, some species have suckers at the tops of the crest, while others (*Loliolus hardwickei, L. affinis*) have a suckerless fused crest.

F. Fin morphology

30. General fin shape (subterminal and round/terminal, longitudinally oval/terminal, rhomboid, or transversely oval/longitudinally oval and trabeculate) - The general shape of the swimming fins on the mantle of cephalopods is highly variable. Fin morphology for the species in this study can be split into four groups. Some outgroup species possess small, round, or kidney-shaped subterminal fins. Some species (*Sepia, Sepioteuthis*) possess fins which extend from almost the anterior edge of the mantle to the posterior tip, and are shaped like half-ovals. *Ctenopteryx* possesses longitudinally oval, trabeculate fins that are rather distinct from the fins of other squids. Most loliginid species have terminal fins whose anterior attachment point is far from the anterior edge of the mantle. These fins are either rhomboid or transversely oval in shape.
31. Anterior fin edge (nearly straight/convex).

32. Posterior fin edge (straight/convex/concave, longer than anterior edge).

G. Sexual morphology

33. Accessory nidamental glands (absent/present).
34. Cutaneous ridge on ventral surface of mantle in males (absent/present) - Mature males of some loliginid species possess a robust, serrated ridge running the length of the ventral midline of the mantle. Males of most loliginid species lack this feature.
35. Male arm II sucker size (normal/proximal suckers enlarged/all suckers enlarged) - Males of particular loliginid species have larger suckers (either proximally, or along the entire length of the arm) on their second (dorsolateral) arm pair than females of similar size of the same species.
36. Male arm III sucker size (normal/proximal suckers enlarged/all suckers enlarged) - See description for character 35.
37. Male right arm IV sucker size (normal/proximal suckers enlarged/proximal suckers reduced) - As described in character 35, males of some loliginid species show enlargement (or reduction) of the proximal suckers of the right arm IV suckers relative to the proximal suckers on the hectocotylus (left arm IV).

H. Spermatophores (Hess, 1987)

38. Spermatophore placement (onto buccal membrane/near left gill on mantle wall/on buccal membrane and left gill/on buccal membrane and right gill) - Clusters of deposited spermatophores can often be found during dissections of females. The location of these clusters varies across species. Females in most species possess a spermatophore receptacle on the buccal membrane near the mouth. In some well-studied species, however, spermatophores have been found attached to the buccal receptacle and to the base of either the left or right gill. In particular species, toughened "spermatophoric pads" can be found on the inside of the mantle cavity near the left gill where spermatophores are attached. Lu *et al.* (1985) reported that some females of *Loliolus noctiluca* also possess spermatophoric pads, and other authors have seen spermatophores placed on the left side of the inner mantle wall in *Loligo opalescens* and *L. pealei* (see Drew, 1911; McGowan, 1954; Fields, 1965). This character needs to be reviewed further, and may prove to be variable across several (or most) loliginid species, potentially limiting its usefulness in cladistic analysis.
39. Spermatophore cement body ratio (oral portion longer than aboral portion/oral and aboral portions approximately equal in length/oral portion smaller than aboral portion) - Many of these data (and data for characters 40 and 41) have been coded directly into the matrix from Hess (1987).
40. Spiral filament in spermatophore (absent/present).
41. Oral component of spermatophore cement body (not divided/divided).

I. Miscellaneous

42. "Conus" (absent/present with edges fused/present, with edges unfused) - In species which possess internal, non-calcified shell remnants (pens or gladii), some possess a "secondary conus" (Toll, 1982) in which the posterior edges of the gladius are fused around the posterior visceral mass to form a cone. In some loliginids, the posterior edges of the gladius are curled ventrally and actually overlap ventrally, but are not fused. Most loliginids possess gladii which show only moderate ventral curling posteriorly (they lack a "conus").
43. Papillae on ink sac of males (absent/present) - Research on the genus *Loliolus* (Lu *et al.*, 1985) has shown that males of two species possess small pores on the ink sac. This characteristic has not been reported in any other loliginid species, and was not found in males of any other species examined in this study.
44. Cornea (absent/present) - The presence or absence of a corneal covering over the eye has been the nominal character separating the oegopsid squids from the myopsid squids.
45. Oviducts (both developed/only left oviduct developed).
46. Muscular septum in mantle cavity (absent/present) - Certain outgroup taxa (*Rossia, Euprymna*) possess a muscular septum dividing the mantle cavity longitudinally into two halves. Loliginids and other taxa in this study lack this feature.
47. Nuchal cartilage (absent/present).
48. Digestive gland (single/paired).

APPENDIX III. Material Examined. Material examined is listed by species name, ingroups first, in alphabetical order. The sex and approximate dorsal mantle length, when known, are listed for each specimen examined. (CAS, California Academy of Sciences; DML, dorsal mantle length; F, female; J, juvenile (sex not determined); M, male; NMNH, United States National Museum of Natural History; U, sex undetermined; UMML, University of Miami Invertebrate Museum).

Ingroup taxa

Alloteuthis africana Adam, 1950 - NMNH 727426 (1 M, 56 mm DML), NMNH BCF Table 6IX 6E-2-218 9-6-63 (2 M, 78 and 71 mm DML), UMML 1757 (1 F, 45 mm DML; 1 M, 58 mm DML).

A. media (Linné, 1758) - NMNH 817475 (3 F, 56, 64, and 67 mm DML; 2 M, 42 and 50 mm DML), UMML 1251.

A. subulata (Lamarck, 1798) - UMML 1252 (2 M, 100 and 101 mm DML), NMNH 817534 (1 F, 70 mm DML).

Loligo beka Sasaki, 1929 - UMML 1209 (1 F, 55 mm DML), UMML 1210 (1 M, 59 mm DML).

L. bleekeri Keferstein, 1866 - NMNH 332905 (1 J, 40 mm DML), UMML 1211 (2 M, 36 and 38 mm DML).

L. budo (Wakiya and Ishikawa, 1921) - UMML 1212 (1 F, 170 mm DML; 1 M, 190 mm DML).

L. chinensis Gray, 1849 - UMML PJ-102 (2 F, 75 and 107 mm DML), UMML PJ-110 (1 F, 92 mm DML).

L. duvauceli Orbigny, 1848 - NMNH 817827 (2 F, 100 and 123 mm DML), NMNH 817829 (1 M, 126 mm DML), NMNH 727560 (1 F, 110 mm DML), NMNH 727561 (2 M, 70 and 93 mm DML), NMNH 817823 (1 M, 66 mm DML), CAS 084583.

L. edulis Hoyle, 1885 - NMNH 814158 (4 M, 127, 133, 136, and 142 mm DML), CAS 030539 (2 M, 99 and 107 mm DML).

L. etheridgei (Berry, 1918) - UMML 1220 (1 F, 90 mm DML; 1 M, 104 mm DML).

L. forbesi Steenstrup, 1856 - NMNH (1 F, 133 mm DML).

L. gahi Orbigny, 1835 - UMML 2087 (1 F, 72 mm DML), UMML

2090 (2 F, 90 and 91 mm DML; 1 M, 69 mm DML).

L. japonica Hoyle, 1885 - NMNH 727551 (2 M, 75 and 77 mm DML), NMNH 332903 (3 M, 58, 70, and 77 mm DML), UMML 1224 (2 M, 61 and 68 mm DML), UMML 1226 (1 F, 60 mm DML).

L. kobiensis Hoyle, 1885 - UMML 31.2203 (1 F, 87 mm DML; 1 M, 76 mm DML).

L. ocula Cohen, 1976 - UMML 1683 (2 M, 53 and 62 mm DML), NMNH 727095 (2 M, 87 and 127 mm DML) (paratypes), NMNH 727096 (1 F, 89 mm DML).

L. patagonica (Smith, 1881) - UMML 1231 (1 F, 83 mm DML).

L. pealei LeSueur, 1821 - NMNH 730069 (2 M, 85 and 95 mm DML), NMNH 730531, NMNH 730183 (1 M, 206 mm DML), NMNH 814169 (1 F, 136 mm DML), NMNH 814191 (1 M, 90 mm DML; 1 J, 83 mm DML).

L. plei Blainville, 1823 - NMNH 574548 (1 M, 105 mm DML), NMNH 576456 (4 M, 146, 154, 195, and 217 mm DML), NMNH 813979 (2 M, 181 and 260 mm DML), NMNH 814288 (1 F, 120 mm DML; 1 M, 105 mm DML), NMNH 814316 (1 M, 198 mm DML), NMNH 814317 (1 M, 213 mm DML), NMNH 814318 (1 M, 197 mm DML), NMNH 814315 (1 M, 163 mm DML), NMNH 574320 (1 M, 169 mm DML), NMNH 574180 (2 M, 215 and 277 mm DML).

L. reesi (Voss, 1963) - UMML 1803 (1 M, 62 mm DML).

L. reynaudi Orbigny, 1845 - UMML 1233 (1 M, 175 mm DML), UMML 1234 (1 M, 95 mm DML).

L. roperi Cohen, 1976 - NMNH 575874 (1 M, 53 mm DML), UMML 933 (1 F, 38 mm DML; 2 M, 41 and 43 mm DML) (paratypes), UMML 1798 (1 M, 55 mm DML), UMML 72777 (1 M, 77 mm DML) (holotype).

L. sanpaulensis Brakoniecki, 1984 - UMML 1813 (2 M, 144 and 150 mm DML) (paratypes).

L. sibogae (Adam, 1954) - NMNH 575813 (1 F, 123 mm DML; 1 M, 139 mm DML).

L. singhalensis Ortmann, 1891 - UMML 31.2323 (1 M, 140 mm DML), UMML 2168 (1 M).

L. sumatrensis Orbigny, 1835 - NMNH 817821 (1 F, 52 mm DML), NMNH 817820 (1 F, 53 mm DML; 2 M, 48 and 50 mm DML).

L. surinamensis Voss, 1974 - UMML 2053 (1 F, 92 mm DML), UMML 31.2023 (2 F, 76 and 88 mm DML).

L. uyii Wakiya and Ishikawa, 1921 - CAS 035049, UMML 1239 (1 F, 94 mm DML; 1 M, 69 mm DML).

L. vossi (Nesis, 1982) - UMML 1259 (2 M, 65 and 78 mm DML).

L. vulgaris Lamarck, 1798 - UMML 1240 (1 M, 210 mm DML), UMML 1241 (1 F, 43 mm DML), UMML 1597 (1, 137 mm DML).

Loliolopsis diomedeae (Hoyle, 1904) - CAS 030492 (2 M, 38 and 41 mm DML), NMNH 576907 (2 F, 90 and 93 mm DML), NMNH 730085, UMML 31.697 (1 F, 102 mm DML), UMML (2 F, 95 and 104 mm DML), UMML 1799 (1 M, 83 mm DML).

Loliolus affinis (Steenstrup, 1856) - CAS 030250 (2 M, 21 and 25 mm DML).

L. hardwickei (Gray, 1849) - CAS 030251 (1 M, 40 mm DML), NMNH 817822.

L. noctiluca Lu, Roper, and Tait, 1985 - NMNH 00813974 (1 F, 68 mm DML; 3 M, 50, 51, and 56 mm DML).

Lolliguncula argus (Brakoniecki and Roper, 1985) - CAS 030252 (2 F, 43 and 43 mm DML; 1 M, 39 mm DML).

L. brevis (Blainville, 1823) - CAS 030491 (2F, 42 and 43 mm DML), NMNH 884122 (1 M, 66 mm DML).

L. mercatoris Adam, 1941- UMML 1244 (1 M), UMML 31.790 (1 M, 15 mm DML), UMML 31.2550 (1 M, 21 mm DML).

L. panamensis Berry, 1911 - CAS 030157 (1 M, 44 mm DML), CAS 030495 (2 F, 86 and 105 mm DML).

Pickfordiateuthis pulchella (Voss, 1953) - UMML 1948 (20 mm DML).

Sepioteuthis australis Quoy and Gaimard, 1832 - NMNH 816311 (1 F, 102 mm DML).

S. lessoniana Lesson, 1830 - CAS 030624 (2 F, 93 and 105 mm DML), NMNH 297637 (2 M, 127 and 166 mm DML), NMNH CH6-7 (1 M, 155 mm DML).

S. loliginiformes (Rüppell and Leuckart, 1828) - NMNH 730575 (1, 17 mm DML).

S. sepioidea (Blainville, 1823) - CAS 030428 (1 M, 72 mm DML), NMNH 576881 (1 M, 101 mm DML), NMNH 9548 (2 M, 99 and 106 mm DML), NMNH 576877 (1 M, 110 mm DML), NMNH 814382 (1 F, 119 mm DML).

Uroteuthis bartschi Rehder, 1945 - CAS 030485 (1 M, 104 mm DML), NMNH 575388 (1 M, 122 mm DML), UMML 1255 (2 F, 119 and 121 mm DML).

Outgroup taxa

Bathyteuthis berryi Roper, 1968 - NMNH 727573 (1 M, 47 mm DML).

Ctenopteryx sicula (Vérany, 1851) - NMNH 728929, NMNH 727721, NMNH 730695 (1 U, 68 mm DML), NMNH 728935 (2 U, 21 and 45 mm DML), NMNH 730696 (1 U, 75 mm DML), NMNH 730697 (1 M, 81 mm DML), NMNH 730698 (1 F, 52 mm DML).

Euprymna moresi (Verrill, 1881) - CAS 021433 (1 F, 31 mm DML; 1M, 33 mm DML).

E. scolopes (Berry, 1913) - CAS 030512 (2 U, 24 and 28 mm DML), CAS 030751 (1 U, 30 mm DML).

Rossia pacifica Berry, 1911 - CAS 030356 (2 F, 30 and 30 mm DML), CAS 081003 (1 U, 50 mm DML).

Sepia aculeata Orbigny, 1848 - CAS 084742 (1 M, 210 mm DML).

Moroteuthis robusta (Dall in Verrill, 1876) - CAS 030111 (partial specimen, total length 9 ft., 7 inches), CAS 035031 (1 U, 300+ mm DML).

Todarodes pacificus (Steenstrup, 1880) - CAS 024414 (2 U, 151 and 155 mm DML), CAS 024415 (1 U, 166 mm DML), CAS 030961, CAS 031020 (1 U, 106 mm DML).

Biochemical study of the population heterogeneity and distribution of the oval squid *Sepioteuthis lessoniana* complex in southwestern Japan

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Abstract: The three taxa of the *Sepioteuthis lessoniana* (Lesson, 1830) complex, AKAIKA, SHIROIKA, and KUAIKA, are genetically and reproductively independent and coexistent without hybridizing along the coast of Ishigaki Island, Okinawa. The present study analyzed 554 specimens of *Sepioteuthis* collected from 11 localities in inshore waters of southwestern Japan to elucidate the distributional patterns and population structures by means of horizontal starch gel electrophoresis at 13 genetic loci encoding for ten enzymes. The results showed that each of the three taxa has a different distributional pattern. SHIROIKA is widely distributed in the tropical to warm temperate regions throughout southwestern Japan. AKAIKA is distributed in the Ryukyu Islands and also probably occurs on the Pacific coast of Honshu, mainland Japan. KUAIKA is limited to the tropical region and its latitudinal distribution suggests a close correlation with water temperature. In SHIROIKA and KUAIKA, significant genetic differences were detected between the specimens from Ogasawara Islands and those from the other localities suggesting the existence of a certain barrier of panmixia between insular localities.

The oval squid, *Sepioteuthis lessoniana* (Lesson, 1830), is a loliginid squid widely distributed throughout the Indian Ocean and the western to central Pacific Ocean (Adam, 1939; Okutani, 1973). In Japan, this squid occurs in inshore waters extending from southern Hokkaido to Okinawa Islands (Sasaki, 1929; Okutani, 1973) and is one of the commercially important squids for neritic fisheries, especially in southwestern Japan (Dotsu *et al.*, 1981; Tsuchiya, 1982; Suzuki *et al*, 1983; Ueta *et al.*, 1992).

On Ishigaki Island, Okinawa, southwestern Japan, fishermen have traditionally distinguished the oval squid into three different groups, namely, AKAIKA, SHIROIKA, and KUAIKA, based on the size, color in freshly-killed condition, and the fishing season and ground (Okutani, 1984; Segawa *et al.*, 1993a, b; Izuka *et al.*, 1994). Izuka *et al.* (1994) carried out allozyme electrophoresis resolving 11 loci in three groups of squids from Ishigaki Island. The results showed that the three groups differed from each other at least among three genetic loci. In addition, three types of egg capsules containing a different number of eggs per capsule were reported from Ishigaki Island (Segawa *et al.*, 1993a, b). Izuka *et al.* (1994) made it clear by allozyme electrophoresis that each group of squid produces different egg capsules: SHIROIKA produces egg capsules containing 4-8 eggs (mode = 6); KUAIKA lays two-egg capsules; and AKAIKA lays egg capsules containing 5-13 eggs (mean = 9.2; SD = 1.2). Further, each type of egg capsule is laid on a different substratum (Segawa *et al.*, 1993a, b; Izuka *et al.*, 1994). These facts indicate that these three groups of *Sepioteuthis lessoniana* along the coast of Ishigaki Island are genetically and reproductively independent despite sympatry. Izuka *et al.* (1994) concluded that these three groups should be regarded as distinct species rather than as infraspecific fractions of a single species, *S. lessoniana.*

To date, allozyme electrophoresis has been a good tool for elucidating interspecific relationships (Augustyn and Grant, 1988; Brierley and Thorpe, 1994; Yokawa, 1994) and cryptic species (Smith *et al.*, 1981; Brierley *et al.*, 1993a; Yeatman and Benzie, 1993; Izuka *et al.*, 1994), and also providing useful information on population structure (*e.g.* Ally and Keck, 1978; Christofferson *et al.*, 1978; Garthwaite *et al.*, 1989; Brierley *et al.*, 1993b). The present study attempts to clarify the population structure and distribution of the three taxa currently referred to the *Sepioteuthis lessoniana* complex in southwestern Japan by allozyme analysis.

MATERIALS AND METHODS

A total of 554 specimens of *Sepioteuthis* were collected from ten localities in inshore waters of southwestern Japan and a single site at Rayong in the Gulf of Thailand by

Table 1. Sampling data for squids used in the present study. Abbreviation of locality as mentioned in tables and figures are in parentheses.

Sampling Locality	Abbreviated Locality Name	N	Date of Collection
Ishigaki Island, Okinawa Pref.	(Ishigaki)	83	May-Oct. 1992
Amami Island, Kagoshima Pref.	(Amami)	22	Feb.-Mar. 1994
Ogasawara Islands, Tokyo	(Ogasawara)	178	June-Oct. 1994
Miyazu City, Kyoto	(Kyoto)	39	Oct. 1992
Sakai, Toyama Pref.	(Toyama)	17	Oct. 1992
Tsuruga, Fukui Pref.	(Fukui)	20	Oct. 1993
Nagato, Yamaguchi Pref.	(Yamaguchi)	36	Feb. 1993
Miura Peninsula, Sagami Bay	(Sagami)	8	July 1994
Hiwasa, Tokushima Pref.	(Tokushima)	102	Aug.-Dec. 1994
Shima, Mie Pref.	(Mie)	45	Aug. 1994
Rayong, Gulf of Thailand	(Thailand)	4	Jan. 1994
	Total	554	

set net, jigging, or scoop net during the period from May 1992 to December 1994 (Fig. 1, Table 1). The squid samples from Ishigaki Island used in the present study were the same as those studied by Izuka *et al.* (1994) with additional genetic data for three loci of *AAT-2**, *G3PDH**, and *PGM**. All the samples were transferred immediately after collection to the laboratory of Tokyo University of Fisheries and kept frozen at -80°C until analysis. Horizontal starch gel electrophoresis was carried out using buccal mass muscle (for details of methods, see Izuka *et al.*, 1994). Three

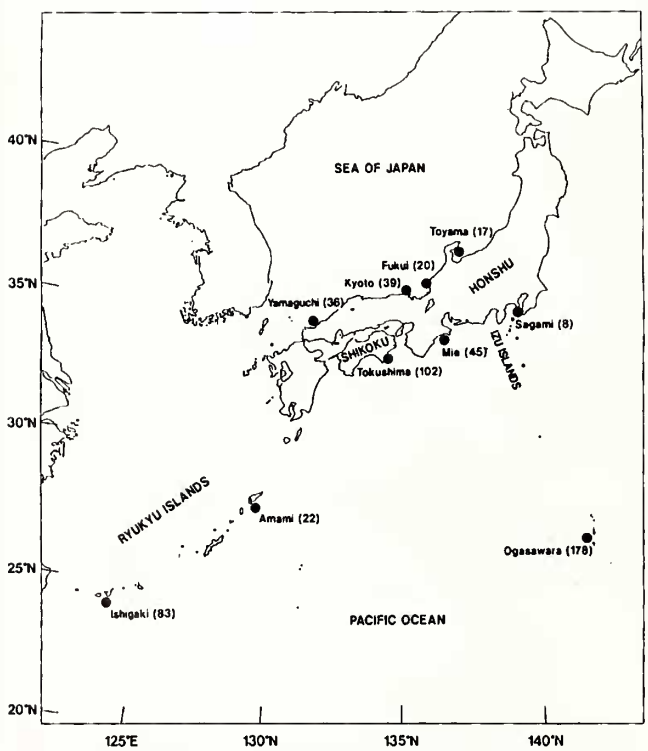

Fig. 1. Sampling localities of the specimens examined in the present study. Numbers in parentheses indicate number of specimens analyzed.

buffer systems described by Numachi (1989) were used for electrode and gel: citrate-N-(3-aminopropyl) morpholine buffer at pH 6 and pH 7 (CAPM6 and CAPM7) and tris-citric acid buffer at pH 8 (CT-8N).

After electrophoresis, the gel was stained for ten enzymes (Table 2). Staining protocols were cited from Hillis and Moritz (1990) except for diaphorase which followed Harris and Hopkinson (1976). Nomenclature of locus and allele follows the guidelines of Shaklee *et al.* (1990). All the samples were classified into AKAIKA, SHIROIKA, and KUAIKA on the basis of asparate aminotransferase genotypes which are taxon-specific (Izuka *et al.*, 1994).

Differences in allele frequencies among localities within every combination of taxa were tested for significance ($P < 0.05$) using the chi square test (Kimura, 1960). Intrapopulational variability was evaluated by the proportion of polymorphic loci (P) (5% level) and heterozygosity (H). However, heterozygosity was not analyzed when the sample size was < 15 specimens for statistical reasons (Nei, 1987). Nei's (1972) genetic distance (D) was calculated from the data of allele frequencies, and the unweighted paired group method of cluster analysis (Sokal and Sneath, 1963) was employed to establish genetic relationships among the squids sampled.

RESULTS

We detected 47 individuals of AKAIKA from three localities, 470 SHIROIKA from 11 localities, and 37 KUAIKA from three localities (Fig. 2). No other kinds of oval squid were found in the present materials. The three taxa could be completely identified by fixed genetic differences at *AAT-1** (Table 3). Taxon-specific alleles were also recognized at *DIA** and *IDHP** in SHIROIKA, and at

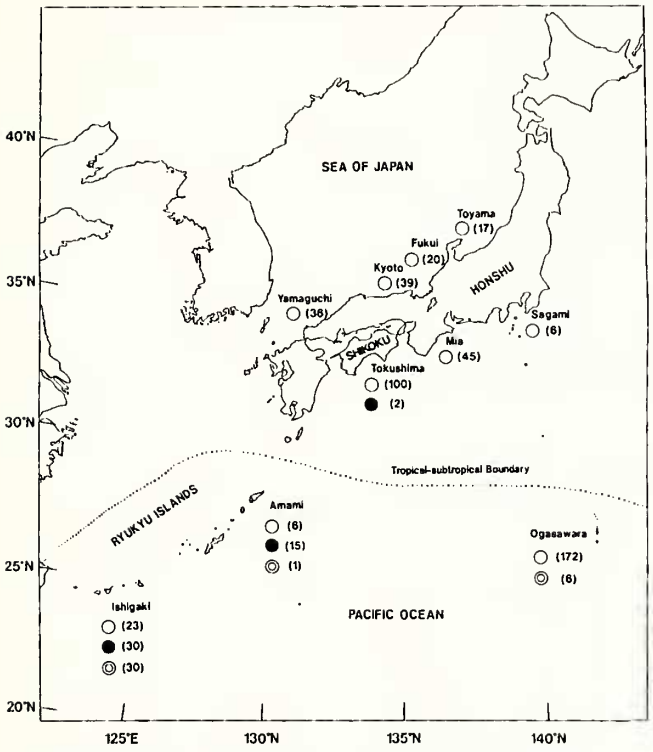

Fig. 2. Distribution of each of the three taxa as detected by electrophoresis. ○, SHIROIKA ; ●, AKAIKA ; ◎, KUAIKA. Numbers in parentheses indicate number of specimens detected.

*MDH-1** and *SORD** in KUAIKA (Table 3).

AKAIKA

AKAIKA squid were found in samples from Tokushima, Shikoku, and Amami and Ishigaki Islands (Fig. 2). No significant difference in allele frequency was detected among these squids, and genetic distances among these sites were close to zero (Fig. 3). All loci were monomorphic except for a single *PGDH** heterozygote from Ishigaki Island (Table 3). These observations indicate that the squids of these three localities are not differentiated from each other and may share a common gene pool.

KUAIKA

KUAIKA was detected from the Ryukyu and Ogasawara Islands, and never observed in the seven samples from inshore waters around Honshu (Fig. 2). No significant genetic difference was detected between the specimens from Amami and Ishigaki Islands indicating that the KUAIKA squid population within the Ryukyu Islands is genetically uniform. However, a significant difference in allele frequency was detected by chi square test at *PGM** between the squids of Ryukyu Islands and Ogasawara Islands. The Ogasawara Islands specimens were fixed for *PGM*a* while only a few heterozygotes from Ishigaki Island exhibited this allele (Table 3). Genetic distance between the populations of Ogasawara Islands and those of Ryukyu Islands was 0.0689 (Fig. 3). Genetic variation was observed only in the squids of Ishigaki Island (Table 3) but the other samples are too small for comparison.

SHIROIKA

SHIROIKA squid were widely distributed through inshore waters around Honshu and from the Ogasawara and Ryukyu Islands, and in the Gulf of Thailand (Fig. 2). Except for the Ogasawara Islands sample, no significant genetic differences were observed among these populations and genetic distances were close to zero (Fig. 3). These facts suggest that SHIROIKA squid from Honshu, Shikoku, Ryukyu Islands, and Thailand could share common gene pool over their 2000 km geographical range. In contrast, slight but significant differences of allele frequencies were detected between the squids of Ogasawara Islands and those

Table 2. List of enzymes, loci examined, and buffers used for electrophoresis.

Enzyme	E.C. No.	Abbreviation	Loci	Buffer
Asparate aminotransferase	2.6.1.1	AAT	*AAT-1**	CAPM6
			*AAT-2**	"
Diaphorase	1.6	DIA	*DIA**	"
Glycerol-3-phosphate dehydrogenase	1.1.1.8	G3PDH	*G3PDH**	CAPM7
Glucose-6-phosphate isomerase	5.3.1.9	GPI	*GPI**	CAPM6
Isocitrate dehydrogenase	1.1.1.42	IDH	*IDH**	CAPM7
Malate dehydrogenase	1.1.1.37	MDH	*MDH-1**	"
			*MDH-2**	"
			*MDH-3**	"
Mannose-6-phosphate isomerase	5.3.1.8	MPI	*MPI**	CT-8N
6-Phosphogluconate dehydrogenase	1.1.1.44	PGDH	*PGDH**	CAPM7
Phosphoglucomutase	5.4.2.2	PGM	*PGM**	CAPM6
Sorbitol dehydrogenase	1.1.1.14	SORD	*SORD**	CAPM7

Table 3. Allele frequency, proportion of loci polymorphic (P), and heterozygosity (H) at 13 genetic loci by locality for three groups.

		AKAIKA			KUAIKA			SHIROIKA										
Locus	Allele	Ishigaki	Amami	Tokushima	Ishigaki	Amami	Ogasawara	Ishigaki	Amami	Ogasawara	Kyoto	Toyama	Fukui	Yamaguchi	Sagmi	Tokushima	Mie	Thailand
*AAT-1**	*a	–	–	–	–	–	–	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	*b	–	–	–	1.00	1.00	1.00	–	–	–	–	–	–	–	–	–	–	–
	*c	1.00	1.00	1.00	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	(n)	(30)	(15)	(2)	(30)	(1)	(6)	(22)	(6)	(171)	(39)	(17)	(18)	(31)	(8)	(100)	(45)	(4)
*AAT-2**	*a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	(n)	(7)	(13)	(2)	(22)	(1)	(3)	(4)	(6)	(79)	(38)	(4)	(1)	(2)	(8)	(93)	(45)	(4)
*DIA**	*a	1.00	1.00	1.00	1.00	1.00	1.00	–	–	–	–	–	–	–	–	–	–	–
	*b	–	–	–	–	–	–	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	(n)	(30)	(15)	(2)	(30)	(1)	(6)	(23)	(6)	(172)	(39)	(17)	(19)	(22)	(8)	(100)	(45)	(4)
*G3PDH**	*a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	(n)	(3)	(15)	(2)	(8)	(1)	(6)	(4)	(6)	(172)	(38)	(17)	(17)	(28)	(8)	(100)	(45)	(4)
*GPI**	*a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.99	1.00	1.00	1.00	1.00	0.99	1.00	1.00
	*b	–	–	–	–	–	–	–	–	–	0.01	–	–	–	–	0.01	–	–
	(n)	(15)	(15)	(2)	(30)	(1)	(6)	(17)	(6)	(171)	(37)	(6)	(18)	(21)	(8)	(100)	(45)	(4)
*IDHP**	*a	–	–	–	–	–	–	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	*b	1.00	1.00	1.00	1.00	1.00	1.00	–	–	–	–	–	–	–	–	–	–	–
	(n)	(30)	(15)	(2)	(30)	(1)	(6)	(23)	(6)	(171)	(39)	(17)	(20)	(36)	(8)	(100)	(45)	(4)
*MDH-1**	*a	–	–	–	1.00	1.00	1.00	–	–	–	–	–	–	–	–	–	–	–
	*b	1.00	1.00	1.00	–	–	–	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	(n)	(30)	(15)	(2)	(30)	(1)	(6)	(23)	(6)	(171)	(39)	(17)	(20)	(36)	(8)	(100)	(45)	(4)
*MDH-2**	*a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	(n)	(30)	(15)	(2)	(30)	(1)	(6)	(18)	(6)	(171)	(39)	(17)	(20)	(36)	(8)	(100)	(45)	(4)
*MDH-3**	*a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	(n)	(25)	(15)	(2)	(30)	(1)	(6)	(12)	(6)	(121)	(39)	(17)	(20)	(35)	(8)	(100)	(45)	(4)
*MPI**	*a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	(n)	(30)	(15)	(2)	(30)	(1)	(4)	(23)	(6)	(163)	(39)	(17)	(19)	(36)	(8)	(100)	(45)	(4)
*PGDH**	*a	–	–	–	–	–	–	–	–	–	–	–	0.02	–	–	0.01	0.03	–
	*b	0.02	–	–	–	–	–	0.06	–	0.53	0.14	0.12	0.13	0.12	–	0.05	0.07	–
	*c	0.98	1.00	1.00	1.00	1.00	1.00	0.93	1.00	0.47	0.82	0.79	0.83	0.86	0.94	0.89	0.82	1.00
	*d	–	–	–	–	–	–	–	–	–	–	0.06	–	–	–	0.04	0.06	–
	*e	–	–	–	–	–	–	–	–	–	0.04	0.03	0.02	0.02	0.06	0.01	0.02	–
	(n)	(30)	(15)	(2)	(30)	(1)	(6)	(23)	(6)	(171)	(39)	(17)	(20)	(3)	(8)	(100)	(45)	(4)
*PGM**	*a	–	–	–	0.15	–	1.00	–	–	–	–	–	–	–	–	–	0.01	–
	*b	1.00	1.00	1.00	0.85	1.00	–	1.00	0.92	1.00	0.99	1.00	0.90	0.97	1.00	0.94	0.94	1.00
	*c	–	–	–	–	–	–	–	0.08	–	0.01	–	0.10	0.03	–	0.05	0.04	–
	(n)	(24)	(15)	(2)	(20)	(1)	(6)	(21)	(6)	(172)	(38)	(17)	(19)	(32)	(8)	(100)	(45)	(4)
*SORD**	*a	–	–	–	–	–	–	–	–	–	0.05	0.06	–	0.02	–	0.03	0.03	–
	*b	–	–	–	–	–	–	–	–	0.03	0.04	0.06	0.06	0.03	–	0.04	0.04	–
	*c	–	–	–	1.00	1.00	1.00	–	–	–	–	–	–	–	–	–	–	–
	*d	1.00	1.00	1.00	–	–	–	1.00	1.00	0.96	0.91	0.87	0.94	0.95	1.00	0.92	0.92	1.00
	(n)	(22)	(15)	(2)	(30)	(1)	(6)	(16)	(6)	(172)	(39)	(16)	(18)	(29)	(8)	(100)	(45)	(4)
P		0.00	0.00	–	0.08	–	–	0.08	–	0.08	0.15	0.15	0.23	0.15	–	0.23	0.23	–
H		0.00	0.00	–	0.02	–	–	0.01	–	0.01	0.03	0.05	0.04	0.02	–	0.03	0.04	–

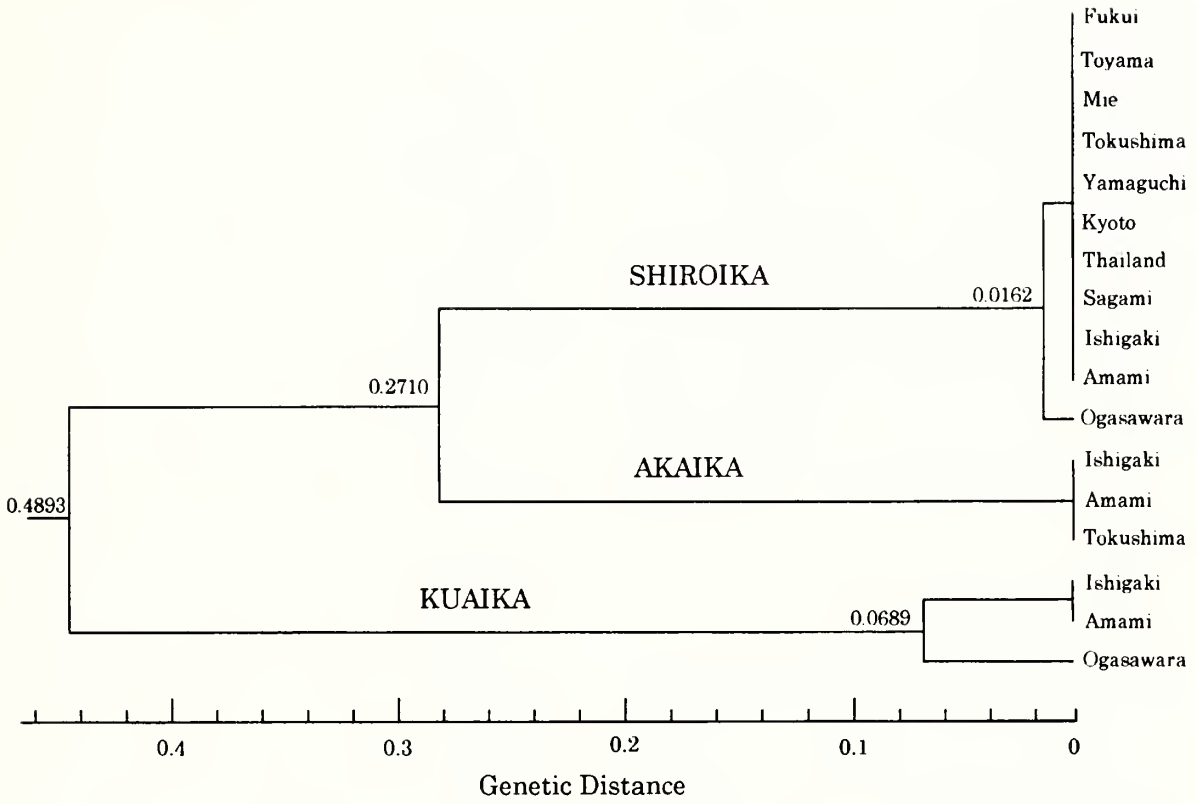

Fig. 3. Biochemical similarity dendrogram based on genetic distance among the squids by locality (for details on localities, see Table 1 and Fig. 1).

of the other localities (D = 0.0162) (Fig. 3).

Genetic variation was detected in all Japanese samples large enough to be tested. Mean heterozygosity (H) of the squids around Honshu was 0.037 (0.023-0.046) and the proportions of polymorphic loci (P) was 0.193 (0.154-0.231) (Table 3). Samples from Ishigaki Island and the Ogasawara Islands were slightly less variable (Table 3).

DISCUSSION

The present study clarified the distributional pattern of each taxon in the *Sepioteuthis lessoniana* complex around southwestern Japan. SHIROIKA is the common widely distributed squid in the tropical to warm temperate region of northwestern Pacific. AKAIKA was detected from Ryukyu Islands and the Pacific coast of Shikoku but probably extends easterly to the Izu Islands as egg capsules containing 6-12 eggs have been observed on deeper bottoms (40-50 m) there (Izuka, unpubl.). KUAIKA was the only taxon restricted to the Ryukyu and Ogasawara Islands and was never found around the main Japanese islands. Amami and Ogasawara Islands are both located in the northernmost part of the tropical region (Briggs, 1974; Nishimura, 1992) and lie near the isotherm of minimum winter temperature of about 20°C (*e. g.* Briggs, 1974; Dall, 1991). Izuka *et al.* (1994) assumed that KUAIKA were confined to the tropical western Pacific, because their egg capsules have been observed only on shallow coral reefs in Ishigaki Island, Okinawa Island, Palau Island, and New Guinea. These facts suggest that KUAIKA is a tropical squid which extends north to the tropical-subtropical boundary (Fig. 3).

Genetic differences appeared in allele frequencies of both SHIROIKA and KUAIKA between Ogasawara Islands and the other sampling localities. This fact may be evidence of a certain barrier against panmixia between these localities. Brierley *et al.* (1993b) found that populations of *Loligo forbesi* Steenstrup, 1856, from the British Isles and the Azores could be considered to be existing in allopatry because of large distance, oceanic depths, and ocean currents between sites. It is unlikely that SHIROIKA and KUAIKA in the Ogasawara Islands maintain sufficient gene flow with those of the mainland and Ryukyu Islands, as the populations of these two areas are separated (more than 1000 km) by the Kuroshio Current. If dispersal of

KUAIKA had taken place between these areas, allele **b* which was recognized as the common allele at *PGM** in the Ryukyu Islands should be detected in the Ogasawara Islands population (Table 3). It suggested that KUAIKA in the Ogasawara Islands is almost genetically segregated from the Ryukyu Islands.

The present study revealed that each of the three taxa of the *Sepioteuthis lessoniana* complex has a different distributional pattern. Within two of these taxa, the populations from the Ogasawara Islands are genetically a little different from those of the other areas sampled. However, the sampling sites in the present study were restricted on the northernmost rim of the western central Pacific. More work including investigations at more southern localities is now required to estimate interpopulational variabilities over the entire geographical range of each of the three taxa.

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Ctenopteryx sicula, a bathypelagic loliginid squid?

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Abstract: *Ctenopteryx sicula* (Vérany, 1851) is an open-eyed, deep-water squid, and as such has traditionally been classified in the suborder Oegopsida (family Ctenopterygidae) along with other families of squid exhibiting these characteristics. *C. sicula* however displays numerous morphological features, including fused axons in the giant nerve fiber system and accessory nidamental glands, found otherwise only within members of the myopsid families Loliginidae and Pickfordiateuthidae. This has lead previous authors to suggest that *Ctenopteryx* species would be more appropriately placed in the suborder Myopsida. Here biochemical genetic evidence is presented which indicates that *C. sicula* is more closely related to several loliginid species than to species of the oegopsid families Histioteuthidae, Ommastrephidae, and Enoploteuthidae. These data, in conjunction with new data on comparative beak morphology, also suggest that *C. sicula* should be considered an oceanic myopsid species.

The squids (Teuthoidea) are generally considered to be divided into two suborders: the Oegopsida which are mainly oceanic, open-water species which lack a corneal membrance covering the eye, and the Myopsida which are mainly near-shore species and which posses a corneal membrane. This membrane has been described as an adaptation preventing ocular damage in shallow, sediment-rich waters of continental shelf areas (Morton and Yonge, 1964). *Ctenopteryx sicula* (Vérany, 1851) (family Ctenopterygidae) is a bathypelagic squid species of cosmopolitan oceanic distribution (Nesis, 1987; Roeleveld *et al.*, 1992). The species lacks an eye-covering corneal membrane and as such is classified in the suborder Oegopsida along with other families of squid exhibiting this characteristic (for example, see Roper *et al.*, 1969; Nesis, 1987). Naef (1923) and Young (1991) have however highlighted a number of morpological features which are at odds with this scheme of classification. *C. sicula* exhibits fusion of nerve axons in the giant fiber system, and has accessory nidamental glands, features which are otherwise found exclusively within the myopsid families Loliginidae and Pickfordiateuthidae. In addition, *C. sicula* has attachments of the fourth buccal connectives similar to those of *Loligo* species, and has suckers on the buccal lappets, exhibits retraction of tentacles into pockets, and has straight, simple funnel locking cartilages, all of which are reminiscent of loliginid morphology (Naef, 1923; Nesis, 1987; Young, 1991). Young (1991) consequently suggested that *C. sicula* could be a squid species related to the loliginids which has become adapted to life in deep water. Here biochemical genetic techniques are used to investigate relationships between *C. sicula* and member species of the families Loliginidae, Histioteuthidae, Ommastrephidae, and Enoploteuthidae in an attempt to determine to which families it is most closely related, and hence with which suborder the species has closet affinity.

Enzyme electrophoresis is well established as a tool for investigation of taxonomy and systematics (Avise, 1974, 1983; Ferguson, 1980; Ayala, 1983; Richardson *et al.*, 1986; Thorpe and Solé-Cava, 1994), and has been applied to a number of cephalopod problems (Smith *et al.*, 1981; Augustyn and Grant, 1988; Brierley and Thorpe, 1994; Yokowa, 1994; Brierley *et al.*, in press). The theoretical basis for the use of electrophoretic data in taxonomic studies is founded on what has become known as the *molecular clock hypothesis* (Thorpe, 1982; Nei,1987). This hypothesis holds that between reproductively isolated groups of organisms molecules such as enzymes, the structures of which are under direct genetic control, diverge at a rate that is stochastically related to the evolutionary time since divergence. The majority of cases employing electrophoresis as a taxonomic aid have addressed questions raised at the level of populations, species, or genera. The technique has however also provided meaningful data for comparisons between species from related families (for example, see Solé-Cava *et al.*, 1992, 1994), and is therefore an appropriate means for investigation of the question in hand.

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MATERIALS AND METHODS

Squid samples

Ctenopteryx sicula (Vérany, 1851) and the oegopsid squid species *Pyroteuthis margaritifera* (Ruppell, 1844), *Histioteuthis bonellii* (Férussac, 1835), and *Todarodes sagittatus* (Lamarck, 1799) were caught from the northeastern Atlantic Ocean in the region of 21°W, 31°N using a multiple-opening, 8 m^2 rectangular mid-water trawl (RMT8) during RRS *Discovery* cruise 194 in August 1990 (see Herring, 1990). *Sepioteuthis lessoniana* Lesson, 1830, was obtained from Indonesian waters, and *Loligo forbesi* Steenstrup, 1856, and *Loligo vulgaris vulgaris* Lamarck, 1798, were caught around the British Isles. Samples of mantle tissue were dissected from each specimen as soon after capture as possible, and immediately frozen (to -80°C in the case of the *Discovery* samples) prior to transportation to Port Erin Marine Laboratory. Date and location of capture, and species sample sizes, are presented in Table 1.

Electrophoresis

Mantle tissue samples from *Ctenopteryx sicula* and the other oegopsid species were assayed during September 1990 using a series of standard electrophoretic techniques which have been described in detail elsewhere (Brierley, 1992; Brierley *et al.*, 1993a, b). The majority of the loliginids had been examined previously using the same experimental protocols, but a group of ten were run again concurrently with the *Discovery* samples in order to provide a reference for relative band migration. Gels were scored immediately after optimum stain development, and genotypes assigned accordingly.

Data analysis

Allele frequency data was analyzed using the FORTRAN program BIOSYS-1 (Release 1.7) (Swofford and Selander, 1981) to calculate unbiased genetic identity *(I)* and distance *(D)* (Nei, 1978) between species pairs, and to construct a dendrogram of *D* using the unweighted pair-grouping arithmetic mean (UPGMA) cluster analysis algorithm (Sneath and Sokal, 1973). The use of the UPGMA method in conjunction with *D* is generally recognized as the best and most accurate procedure for phylogentic tree reconstruction from electrophoretic data (Nei, 1987). This is partly because *D* can be subject to large stochastic errors (especially if calculated with small numbers of loci), and the procedure of distance-averaging used in UPGMA reduces this error considerably (Nei, 1987).

RESULTS

Seventeen enzyme stain systems developed successfully for all species, revealing the presence of 18 common putative enzyme loci. The group of ten loliginids run concurrently with *Ctenopteryx sicula* and the oegopsid species resolved identically here as previously (Brierley *et al.*, 1993a, 1995; Brierley and Thorpe, 1994). A slight reduction in band intensity was apparent, but band migration and definition remained entirely unaffected by frozen (-26°C) storage. Allele frequencies at the 18 clearly resolving enzyme loci are given for each species in Table 2. Inspection of Table 2 reveals *Histioteuthis bonellii*, *Todarodes sagittatus*, and *Pyroteuthis margaritifera* exhibiting very low levels of mean heterozygosity per locus, a phenomemon which is a seemingly recurrent theme in studies of squid biochemical genetics (see review in Brierley *et al.*, 1993a). Although this ubiquitous feature of low intraspecific genetic variability can cause problems for the use of electrophoretic data in squid population studies (see Brierley *et al.*, 1993b, 1995), taxonomic studies benefit because banding patterns remain simple, aiding interpretation and greatly reducing the likelihood of error (Garthwaite *et al.*, 1989). Mean *I* and *D* between all species pairs are given in Table 3. These unbiased estimates are most appropriate for small sample sizes, especially, as here, when observed heterozygosity levels are low. *D* is purported to be stochastically linear with evolutionary time (Nei, 1987; Thorpe, 1989), and varies between a theoretical minimum of zero in comparison of genetically identical individ-

Table 1. Sample size, and date and location of capture of species examined.

Species	Sample size	Date of capture	Location of capture
Ctenopteryx sicula	4	August 1990	Northeastern Atlantic Ocean
Pyroteuthis margaritifera	11	August 1990	Northeastern Atlantic Ocean
Histioteuthis bonellii	14	August 1990	Northeastern Atlantic Ocean
Todarodes sagittatus	2	August 1990	Northeastern Atlatnic Ocean
Sepioteuthis lessoniana	5	April 1988	South Alas Strait, Indonesia
Loligo forbesi	20	October 1989	Isle of Man, British Isles
L. vulgaris vulgaris	20	October 1989	Plymouth, United Kingdom

Table 2. Allele frequencies at the 18 clearly resolving enzyme loci.

Locus	Allele	*Ctenopteryx sicula* N = 4	*Loligo forbesi* N = 20	*L. v. vulgaris* N = 20	*Sepioteuthis lessoniana* N = 5	*Pyroteuthis margaritifera* N = 11	*Histioteuthis bonnellii* N = 14	*Todarodes sagittatus* N = 2
aGpdh	A	0	0	0	0	0	1	0
	B	0.125	0	0	0	0	0	0
	C	0.875	0	0	0	0	0	1
	D	0	0	0.9	0	0	0	0
	E	0	1	0.1	0	0	0	0
	F	0	0	0	1	1	0	0
Mdh- 1	A	0	1	1	0	0	0	0
	B	0	0	0	1	0	0	0
	C	0.875	0	0	0	0	0	0
	D	0	0	0	0	1	0	1
	E	0.125	0	0	0	0	1	0
Mdh-2	A	0	0	0	0	0	1	0
	B	1	0	0	1	0	0	0
	C	0	1	1	0	0	0	0
	D	0	0	0	0	0	0	1
	E	0	0	0	0	1	0	0
Me	A	0	0	0	1	0	0	0
	B	0	1	1	0	0	0	0
	C	0	0	0	0	1	0	0
	D	1	0	0	0	0	0	0
	E	0	0	0	0	0	1	0
	F	0	0	0	0	0	0	1
Idh	A	0	0	0	0	0	0	1
	B	0	0	0	0	0	1	0
	C	1	0	0	1	0	0	0
	D	0	0.95	1	0	0	0	0
	E	0	0.05	0	0	1	0	0
Pgdh	A	0	0	0	1	0	0	0
	B	0	0	1	0	0	0	0
	C	0	0.025	0	0	0	0	0
	D	0	0.975	0	0	0	0	0
	E	0	0	0	0	1	0	0
	F	1	0	0	0	0	0	0
	G	0	0	0	0	0	1	0
	H	0	0	0	0	0	0	1
Gopdh	A	0	0	0	0	1	0	0
	B	0	0	0	0	0	0	1
	C	0	1	0	1	0	0	0
	D	0	0	1	0	0	0	0
	E	0	0	0	0	0	1	0
	F	1	0	0	0	0	0	0
Gapdh	A	0	0	0	0	1	0	0
	B	0	0	0	0	0	0	1
	C	0	1	0	0	0	0	0
	D	0	0	0	1	0	0	0
	E	1	0	1	0	0	0	0
	F	0	0	0	0	0	1	0
Sdh	A	0	0	0	0	0	1	0
	B	0	0	0	0	1	0	0
	C	1	0	0	0	0	0	0
	D	0	1	0	0	0	0	0
	E	0	0	0	1	0	0	1
	F	0	0	1	0	0	0	0
Pep-A	A	1	0	0	0	0	0	0
	B	0	1	0	0	0	0	0
	C	0	0	1	0	0	0	0
	D	0	0	0	0	0	0	1
	E	0	0	0	1	0	0	0
	F	0	0	0	0	1	0	0
	G	0	0	0	0	0	1	0

(Continued)

Table 2. Continued.

Locus	Allele	*Ctenopteryx sicula* N = 4	*Loligo forbesi* N = 20	*L. v. vulgaris* N = 20	*Sepioteuthis lessoniana* N = 5	*Pyroteuthis margaritifera* N = 11	*Histioteuthis bonnellii* N = 14	*Todarodes sagittatus* N = 2
Ck	A	0.125	0	0	0	0	0	0
	B	0.875	0	0	0	0	0	0
	C	0	1	1	0	0	0	0
	D	0	0	0	1	0	0	0
	E	0	0	0	0	1	1	1
Fum	A	0	0	0	0	0	0	1
	B	0	1	0	0.5	0	0	0
	C	1	0	0	0	0	0	0
	D	0	0	0	0	1	0	0
	E	0	0	1	0	0	0	0
	F	0	0	0	0.5	0	0	0
	G	0	0	0	0	0	1	0
Mpi	A	0	0	0	0.5	0	0	0
	B	0	0	0	0	0	0	1
	C	0	0	0	0.5	0	0	0
	D	0	0	1	0	0	0	0
	E	1	0.975	0	0	0	0	0
	F	0	0.025	0	0	1	1	0
Gpi	A	0	0	0	0	0	0	1
	B	0	0	0	0	1	0	0
	C	1	0	0	0	0	0	0
	D	0	1	1	0	0	0	0
	E	0	0	0	1	0	0	0
	F	0	0	0	0	0	1	0
Ald	A	0	1	1	1	0	1	1
	B	1	0	0	0	0	0	0
	C	0	0	0	0	1	0	0
Odh	A	0	1	0	1	0	0	0
	B	1	0	1	0	1	0	0
	C	0	0	0	0	0	1	0
	D	0	0	0	0	0	0	1
Ldh	A	1	0	0	0	0	0	0
	B	0	1	0.825	0	0	0	0
	C	0	0	0	0	1	0	0
	D	0	0	0	1	0	0	0
	E	0	0	0.175	0	0	0	0
	F	0	0	0	0	0	1	0
	G	0	0	0	0	0	0	1
Sordh	A	0	0.125	0	0	0	0	0
	B	0	0.85	0	1	0	0	0
	C	0	0.025	0	0	0	0	0
	D	0	0	1	0	0	0	0
	E	1	0	0	0	0	0	0
	F	0	0	0	0	1	0	0
	G	0	0	0	0	0	0	1
	H	0	0	0	0	0	1	0

Table 3. Matrix of unbiased genetic identity *(I)* (above diagonal) and distance *(D)* (below diagonal) values between all species pairs.

Species	1	2	3	4	5	6	7
1 *Ctenopteryx sicula*	****	0.056	0.115	0.117	0.057	0.007	0.05
2 *Loligo forbesi*	2.881	****	0.449	0.253	0.004	0.058	0.056
3 *L. vulgaris vulgaris*	2.162	0.800	****	0.058	0.056	0.056	0.056
4 *Sepioteuthis lessoniana*	2.144	1.375	2.845	****	0.057	0.057	0.115
5 *Pyroteuthis margaritifera*	2.869	5.468	2.877	2.859	****	0.111	0.111
6 *Histioteuthis bonnellii*	4.949	2.853	2.877	2.859	2.197	****	0.111
7 *Todarodes sagittatus*	3.003	2.877	2.877	2.165	2.197	2.197	****

uals, and infinity when the pair of taxa under consideration exhibits no common genetic characters. A UPGMA dendogram of *D* (Fig. 1) shows *Ctenopteryx sicula* clustering more closely with the branch encompassing the loliginid species than to the branch containing the oegopsid families Histioteuthidae, Ommastrephidae, and Enoploteuthidae.

DISCUSSION

In contrast to electrophoretic studies of population structuring, in which large sample sizes are required, taxonomic studies such as the one reported here can be successfully conducted using only very small numbers of individuals from each taxa if large numbers of enzyme loci are studied (see for example Richardson *et al.*, 1986). The comparatively small sample sizes of *Ctenopteryx sicula, Todarodes sagittatus,* and *Sepioteuthis lessoniana* are unlikely to be a major source of inaccuracy in calculation of *D*, or, consequently, in reconstruction of the phylogenetic tree shown in Fig. 1. Numbers of animals used have negligible effects upon the errors of genetic distance, and even sample sizes as small as one will give acceptable distance estimates (see Nei, 1978; Gorman and Renzi, 1979; Thorpe, 1982). This fact is well illustrated by a reconstructed phylogeny of the family Ommastrephidae (Yokowa, 1994) which is based on electrophoretic data gathered from 16 species with a mean sample size of less than two.

Yeatman and Benzie (1993) used *Sepioteuthis lessoniana* as an outgroup in a study of cryptic speciation in *Loligo* from Australian waters. They reported the species differing completely from all *Loligo* taxa investigated at eight of the 11 loci they resolved, and consequently reported a *D* value of > 4 between species. Such a high value is far larger than would normally be expected between members of confamilial genera (Thorpe, 1982). We have assayed six (*Ak, Gpi, Idh, Mdh-1, Mdh-2,* and *Mpi*) of these eight loci here and elsewhere (Brierley, 1992; Brierley *et al.*, 1993b, in press) and similarly find alleles unique to *Sepiotheuthis lessoniana* at these loci. However this and our previous analyses have included data from a number of other loci in addition to the 11 investigated by Yeatman and Benzi (1993), and our estimate of *D* between *Loligo* species and *Sepioteuthis lessoniana* of the order of 1.4 is therefore likely to be the more accurate. This large discrepancy between studies highlights the possibility of substantial interlocus errors on measures of genetic distance (Thorpe, 1979, 1982), and emphasizes the importance of screening large numbers of loci.

The phylogenetic tree reconstructed using UPGMA analysis of *D* between all species pairs (Fig. 1) comprises two main branches, one containing member species of the suborder Oegopsida, and the other containing myopsid species. *Ctenopteryx sicula* clusters more closely with the Myopsida, and genetic data presented here are therefore in agreement with morphological evidence (Naef, 1923; Young, 1991) suggesting the species is related to *Loligo. C. sicula* clusters with other myopsid species at a level of *D* which lies within the practical limits of the measure. The *D* value of about 2.9 exhibited here between *C. sicula* and *Loligo forbesi* (see Table 3) is less than the value of $D = 3$, beyond which the measure diverges from linearity (Thorpe, 1989). This limit arises because *D* appears to suffer a "saturation" effect (Thorpe, 1982): a maximum of one nucleotide substitution per locus can be detected electrophoretically, and practical limitations impose a resolution threshold on

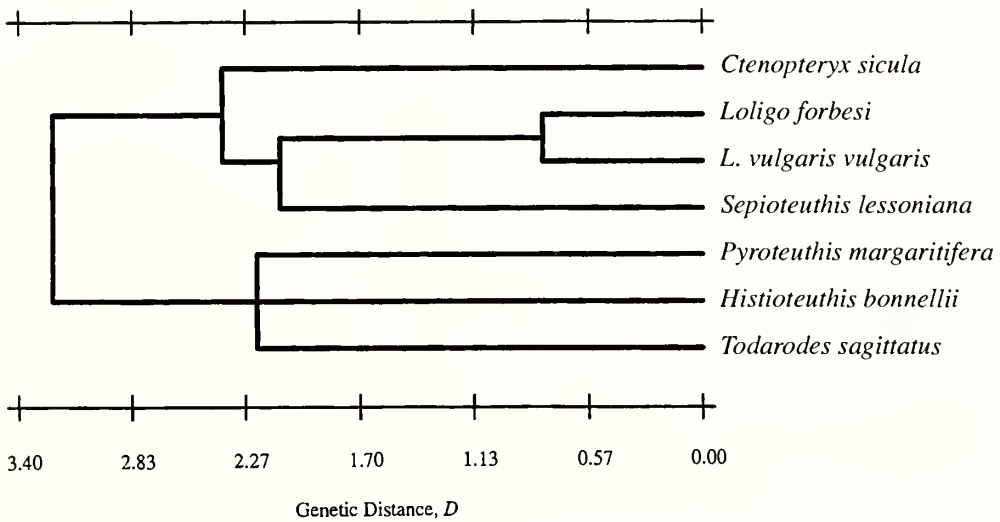

Fig. 1. UPGMA dendrogram of Nei's (1978) unbiased genetic distance *(D)* between all species pairs.

the number of discrete alleles which can be electrophoretically distinguished. Using data gathered from a survey of around 100 loci between species pairs from different major taxa, Thorpe (1982) found an approximate 5% coincidental identity between quite unrelated taxa (I in the order of 0.05, $D = -\log_e I$). The similarity between *C. sicula* and the loliginid species studied here is clearly greater than this 5% similarity. The level at which the oegospid and myopsid species cluster is high, but this does not detract from the validity of the observation that members of the Myopsida and Oegopsida cluster as two discrete entities, or that *C. sicula* is more closely related to loliginids than to any of the oegospid families studied here. We do not attempt to place an exact time of divergence on any of the branch points in Fig. 1, because the absolute relationship between genetic distance and evolutionary time is questionable (Sarich, 1977; Lessios, 1979; Thorpe, 1982; Smith and Coss, 1984). For most taxonomic purposes however, as here, the problem of absolute nonlinearity of D with time is unimportant because only relative values are needed (Thorpe, 1982). As long as genetic distance is approximately linear with time, the relative evolutionary relationships among organisms can be determined (Nei,1987).

Ctenopteryx sicula is positioned within the branch of the phylogenetic tree (Fig. 1) containing *Loligo forbesi* and *L. vulgaris vulgaris* beyond the junction at which *Sepioteuthis lessoniana* diverges from the genus *Loligo*. *Sepioteuthis* has been described, on morphological and distributional grounds, as a Tethyan relict genus which split off from all other loliginids before the closing of the Tethys Sea (Brakoniecki, 1986). *C. sicula* would therefore appear to have diverged from the loliginid line at some time before this event. Naef (1923) suggested that *C. sicula* should be considered the earliest independent branch of the stem of the higher Oegopsida. Genetic data from the present study summarized in the phenogram in Fig. 1 go some way to support this assertion, although further electrophoretic analysis of *Pickfordiateuthis pulchella* Voss, 1953, would be necessary before genetic and morphological data could be viewed in complete congruence. It would seem appropriate therefore to adopt the viewpoint of Young (1991), who has proposed that *C. sicula* may be considered as a teuthoid related to loliginids which has become adapted to a deep-water, oceanic life. Young (1991) has also argued that, despite the fact that the name is inappropriate for a squid exhibiting the open-eyed condition, it would be desirable to classify *Ctenopteryx* species with the families Loliginidae and Pickfordiateuthidae in the suborder Myopsida.

The numerous similarities between *Ctenopteryx* and *Bathyteuthis* species have been well described (Roper, 1969; Nesis, 1987), and more recent analyses of comparative beak morphology have further highlighted the affinity among *Loligo, Ctenopteryx,* and *Bathyteuthis* species (Roper and Clarke, unpub. data). It would be of additional interest to examine *Bathyteuthis* species electrophoretically, as it remains possible that these species similarly fail to comply with the broad categorization of an open-eyed, open-ocean suborder, contrasting entirely with a distantly related shelf-inhabiting group. Our data suggest that the character of the presence or absence of a corneal membrane may not be an infallible guide for distinguishing the two major systematic groupings of squid.

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Biogeography of *Octopus* species (Cephalopoda: Octopodidae) from southeastern Australia

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Abstract: Seven species of inshore, benthic octopuses have been recorded from temperate waters off southeastern Australia. Studies on aspects of reproductive biology show that five species (*Octopus berrima* Stranks and Norman, 1993; *O. bunurong* Stranks, 1990; *O. kaurna* Stranks, 1990; *O. pallidus* Hoyle, 1885; and *O. superciliosus* Quoy and Gaimard, 1832) produce large eggs (8-14 mm long) in low numbers (10s or 100s), and have hatchlings that immediately adopt a benthic existence. Another two species (*O. maorum* Hutton, 1880, and *O. warringa* Stranks, 1990) produce small to medium eggs (2-7 mm long) in high numbers (1000s), with juveniles that are temporarily planktonic before settling out to the benthos. The distribution of these species is influenced by regional oceanographic factors such as the Leeuwin Current, West Wind Drift, and East Australian Current. The five species with direct development have limited means for dispersal, and are restricted in distribution to waters off southeastern Australia; the other two species with indirect development have the potential for long-range dispersal through the waters off southeastern Australia, the Tasman Sea, and New Zealand.

The family Octopodidae is typically characterized by the inshore, benthic octopus that possesses a saccular mantle and eight arms. The best-documented genus in the family is *Octopus*, which has a distribution in all coastal regions except for Arctic and Antarctic areas (Nesis, 1987).

There is a rich and diverse octopodid fauna in tropical to cold-temperate waters of the Australasian continental shelf region (Lu and Dunning, in press; Stranks, in press). During the past decade, the systematics of the regional fauna have been reviewed and revised by several workers, for example, the tropical fauna of Australia by Norman (1992a, b; 1993a, b, c), the cold temperate fauna of Australia by Stranks (1988a, b; 1990) and Stranks and Norman (1993), and the fauna of New Zealand by O'Shea (1990). These studies recognized 19 valid species of *Octopus* from Australia, and eight species from New Zealand. In addition, these works established distributional ranges for most of the valid species, and reported data on general biology where available.

Wilson and Allen (1987) included a general summary of molluscan biogeography from Australia. Lu and Phillips (1985) and Lu and Dunning (in press) have summarized available information on the distribution and biogeography of Australian cephalopods, but there has been little information published on the biogeography of octopodids from the region. Recent taxonomic work on the southeastern Australian fauna has facilitated a biogeographic study (see Stranks, 1988a). The present paper reports on the distribution of *Octopus* in this region related to biological and oceanographic factors.

METHODOLOGY

This study was based on a review of octopus specimens from southern Australian and New Zealand localities, held in collections in Australian museums: The Australian Museum (Sydney) (AM); Museum of Victoria (Melbourne) (NMV); Queen Victoria Museum and Art Gallery (Launceston) (QVM); South Australian Museum (Adelaide) (SAM); and Tasmanian Museum and Art Gallery (Hobart) (TMH). Additional material was examined from collections in New Zealand institutions: Canterbury Museum (Christchurch) (CMNZ); Museum of New Zealand (Wellington) (MONZ); and the Otago Museum (Dunedin) (OMNZ). Other abbreviations used are: CL, capsule length of mature ovarian or spawned egg; ML, mantle length; and TL, total length.

RESULTS

Seven valid species of octopuses have been recorded from southeastern Australian waters (Table 1).

Octopus berrima Stranks and Norman, 1993, is one of the most common *Octopus* species in southeastern Australia, occurring from the central Great Australian Bight (about 132°E) to Twofold Bay, New South Wales (about 37°S), including Bass Strait and Tasmania (Stranks and Norman, 1993). This species produces large eggs (10-14 mm CL) that are attached singly to the substratum (see vouchers NMV F52511 and F77664), and large hatchlings (4-5 mm ML) (see voucher NMV F77665). Mature females of *O. berrima* were recorded by Tait (1980) [as *O. australis* Hoyle, 1885] brooding clutches of 52-129 eggs.

Octopus bunurong Stranks, 1990, is distributed in southeastern Australia from the central Great Australian Bight (about 132°E) to southern New South Wales (about 37°S), including Bass Strait and northern Tasmania (Stranks, 1988a; 1990). Females have medium-sized mature ovarian eggs of 8-10 mm CL (see voucher NMV F53221). Method of egg attachment, clutch size, and hatchling size are unknown.

Octopus kaurna Stranks, 1990, is recorded in southeastern Australia from the central Great Australian Bight (about 132°E) to southern New South Wales (about 37°S), including Bass Strait and northern Tasmania (Stranks, 1988a; 1990). Females have mature ovarian eggs of 9-11 mm CL (see voucher NMV F1628). Method of egg attachment, clutch size, and hatchling size are unknown.

Octopus maorum Hutton, 1880, is a common species widely distributed in southeastern Australia and New Zealand (Fig. 1). The species is recorded on continental-shelf and upper-slope regions in southeastern Australia, from the central Great Australian Bight (about 132°E) to central New South Wales (about 33°S), and also in New Zealand from the North and South islands, and Chatham, Stewart, Auckland, and Campbell islands (Dell, 1952; Stranks, 1988a). The species produces medium-sized eggs (6-7 mm CL) that are attached singly to the substratum (see vouchers SAM D17981 and OMNZ A.'44.22). Batham (1957) reported a female *O. maorum* from New Zealand with a clutch of approximately 7000 eggs, and described morphology of the paralarvae (6.7-7.6 mm TL). Additional details of planktonic hatchling morphology and size (4.3-4.5 mm ML) were given by Hochberg *et al.* (1992). Larger planktonic hatchlings of 13-17 mm ML are also recorded from off eastern Tasmania (see vouchers NMV F77607-F77610).

Octopus pallidus Hoyle, 1885, is another common species in southeastern Australia (Fig. 2), recorded from continental-shelf and upper-slope regions, ranging from the central Great Australian Bight (about 132°E) to central New South Wales (about 33°S) including Bass Strait and Tasmania (Stranks, 1988a, b). One female was observed with a spawned clutch of 270 large eggs (11-13 mm CL) that were attached singly to the substratum (see voucher NMV F52502). The species produces large hatchlings (5-6 mm ML) (see voucher NMV F31563).

Octopus superciliosus Quoy and Gaimard, 1832, is recorded in southeastern Australia from the central Great Australian Bight (about 133°E) to Twofold Bay, New South Wales (about 37°S), including Bass Strait (Stranks, 1988a). The species produces large eggs (8-11 mm CL) that are attached singly to the substratum, and large hatchlings (4-5 mm ML) (see voucher NMV F59403). Clutch size is unknown.

Octopus warringa Stranks, 1990, has a wide distribution in southeastern Australia and New Zealand; it is recorded from the central Great Australian Bight (about 125°E) to eastern Victoria (about 38°S), and also in New Zealand from the North and South islands, and Stewart Island (Dell, 1952; Stranks, 1988a; 1990). The species produces small eggs (2-3 mm CL) that are attached in festoons to the substratum (see vouchers NMV F53216 and AM C92051). Brough (1965) reported on a female *O. warringa*

Table 1. Mature female size, egg size and number, hatchling type, and general distribution of *Octopus* species in southeastern Australian waters (*, measured from mature ovarian eggs; **, estimated from number of ovarian eggs).

Species	Female size at maturity (mm ML)	Egg size (mm CL)	Egg number	Hatchling size (mm ML)	Geographical distribution
Small to Intermediate Egg Species with Planktonic Hatchlings					
Octopus maorum	90-255	6-7	7000	4-5	SE Australia-New Zealand
O. warringa	15-30	2-3	1000	2-3	SE Australia-New Zealand
Large Egg Species with Benthic Hatchlings					
O. berrima	30-90	10-14	50-130	4-5	SE Australia
O. bunurong	45-50	8-10*	50-100*	?	SE Australia
O. kaurna	40-85	9-11*	50-100**	?	SE Australia
O. pallidus	50-130	11-13	270	5-6	SE Australia
O. superciliosus	10-30	8-11	50-100**	4-5	SE Australia

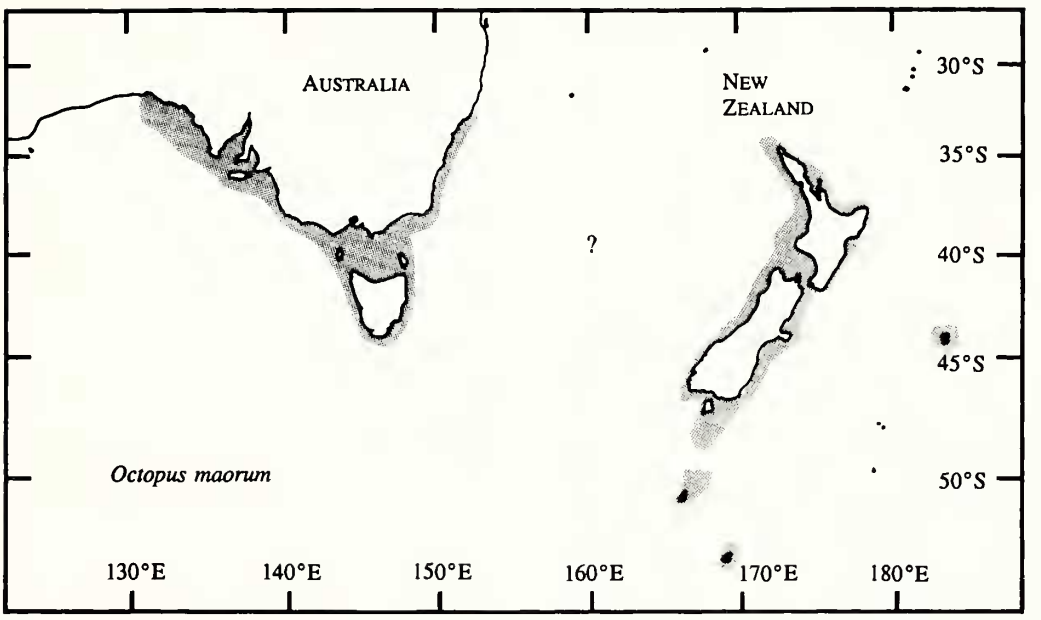

Fig. 1. Generalized geographical distribution of small- to medium-egg sized species around the southeastern coast of Australia, and around New Zealand and adjacent islands (based on the distribution of *Octopus maorum*).

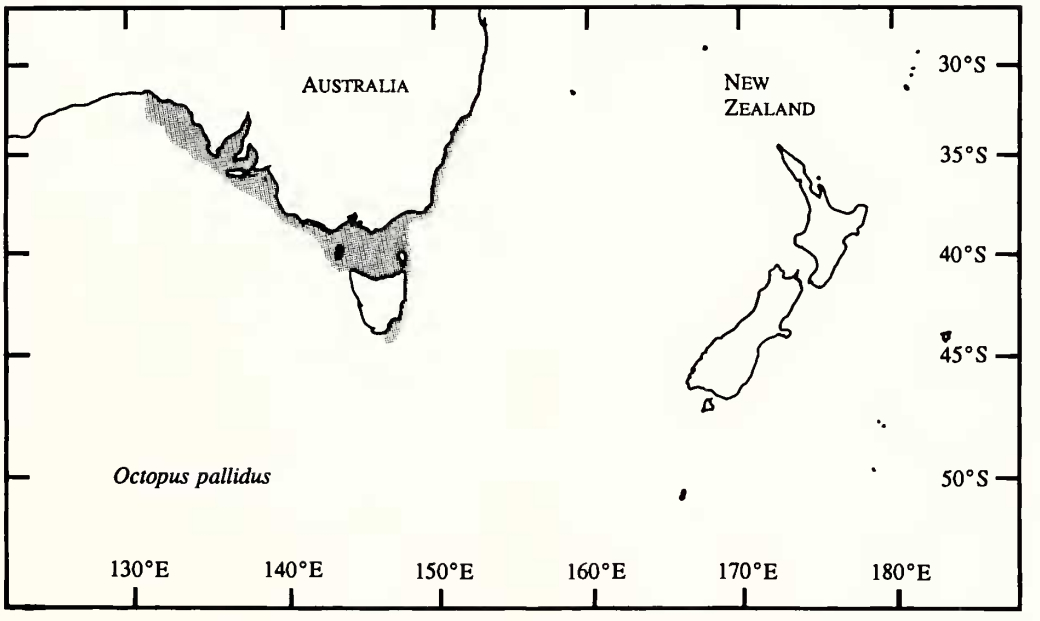

Fig. 2. Generalized geographical distribution of large egg sized species around the southeastern coast of Australia (based on the distribution of *Octopus pallidus*).

[as *Robsonella australis* (Hoyle, 1885)] brooding a clutch of about 1000 eggs, and on the morphology of the resulting hatchlings (3.6-4.0 mm TL). Further details of planktonic hatchling morphology and size (2.2-2.3 mm ML) were given by Hochberg *et al.* (1992).

DISCUSSION

The seven species of *Octopus* recorded in the present study have a localized distribution in the inshore temperate waters of southeastern Australia, and additionally with two cases, in New Zealand. The generalized distributional pattern of this species group on the Australian continent is from the Great Australian Bight to the southern or central New South Wales coast, including Bass Strait and the Tasmanian coastline. Those species also occurring in New Zealand are generally distributed around the North and South islands, and several of the smaller offshore islands. The author has not identified any of the seven *Octopus* species in areas outside these geographic limits, and on the basis of studies to date, all the species are considered endemic to the southeastern Australian and New Zealand region. This endemism at the species level corresponds well with the very high level of endemism (probably over 95%) of the southern Australian Mollusca in general (Wilson and Allen, 1987).

Adult inshore octopuses of southeastern Australia and New Zealand have a rather restricted distribution over the continental shelf and upper continental slope, from the intertidal zone to water depths of about 500 m. Adult animals can actively swim for short periods, but do not appear to migrate over extended ranges along the continental shelf, nor venture deep down the continental slope. Deep-sea trawling of mid- to lower continental slope benthos has failed to capture any of the mentioned species. Deep-sea regions could act as physical barriers to migration of these species. In this region, the Indian, Southern, and Pacific Oceans, as well as the Tasman Sea, can be considered as such oceanic barriers. Conversely, shallower stretches of water such as Bass Strait (between the Australian mainland and Tasmania) with water depths of less than 200 m do not appear to present any barrier to migration.

Apart from bathymetric isolation, other factors can influence the distribution of *Octopus* species along the continental shelf at the peripheries of their ranges. Several of the southeastern Australian species are restricted to waters east of the head of the Great Australian Bight (approximately 132°E). A local geographic feature that might present a barrier to migration of some species further west of this longitude is the cliffed Nullarbor coastline. From Eucla, Western Australia (129°E), to the head of the Great Australian Bight (132°E) is a long stretch of coast with very steep cliff frontage, extensive wave-cut platforms, and strong to extreme wave actions (Womersley and Edmonds, 1958). Whether *Octopus* species can survive in such a harsh environment is uncertain at present. Locality records for this area of the range are unfortunately sparse, so this distributional limit could to some extent be an artifact due to lack of sampling. Geographical features that might limit migration at the periphery of species' distributional ranges in eastern Australia are not obvious. Many of the *Octopus* species of southeastern Australia have ranges of distribution that extend along the eastern Australian coast as far northwards as southern or central New South Wales (32-37°S), but such distributions could be the result of hydrological rather than coastal topographical factors. This could also be reflected in the distributions of *Octopus* species around the coasts of New Zealand.

An important factor affecting the distribution of *Octopus* species in southeastern Australia and New Zealand appears to be water temperature. This temperate region has characteristic temperature profiles, described by Womersley and Edmonds (1958) and Knox (1975), that are distinct from those of neighboring areas. Distributional patterns of *Octopus* of southeastern Australia and New Zealand could be correlated with temperature regimes over these regions. Species in these temperate waters appear to tolerate temperature ranges from 10-17°C during the winter period, and 13-23°C through the summer season.

Hydrology of the temperate waters of southeastern Australia affects the distribution of inshore *Octopus* species, particularly where there is a meeting of cold and warm currents, marked by a sharp change in water temperature over a relatively short distance. The generalized temperate distribution of inshore *Octopus* species is confined in southeastern Australia, from about the center of the Great Australian Bight (130-134°E) to central or southern New South Wales (32-37°S), including Bass Strait and Tasmania. These distributional boundaries along the continental shelf in southeastern Australia could be correlated with effects of the Leeuwin Current in the west, and the East Australian Current to the east (Fig. 3). In summary, the Leeuwin Current carries warmer subtropical water around the southwestern coast of Western Australia to at least as far as the head of the Great Australian Bight (130°E) (Rochford, 1986), and can transport warm-water tropical fauna into the Bight (Maxwell and Cresswell, 1981). The East Australian Current carries warmer subtropical waters along the coast of eastern Australia, and can transport warm tropical fauna at least as far as central New South Wales (32-34°S) (Ekman, 1953).

Seawater salinity can be eliminated as a factor affecting distribution of *Octopus* species in southeastern Australia and New Zealand, as the surface salinity profiles are relatively uniform throughout this temperate region

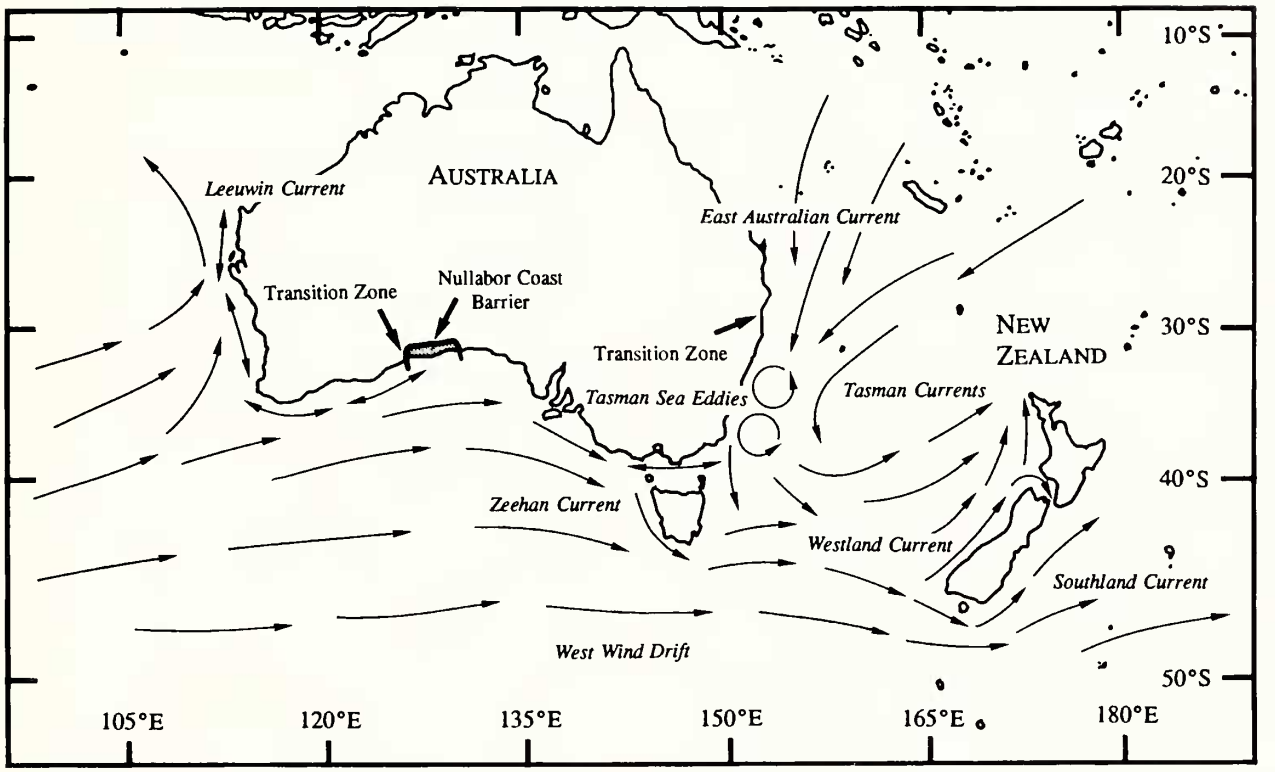

Fig. 3. Schematic chart of principal sea-surface currents influencing distribution of inshore benthic octopuses in the southeastern Australian and New Zealand region.

(Knox, 1975; Bunt, 1987).

Two alternate patterns of geographical distribution and reproductive behavior were noted. Two of the seven *Octopus* species have an extended distribution in southeastern Australian and New Zealand waters. The two taxa involved, *O. maorum* and *O. warringa*, are coincidentally the only species that produce small to intermediate sized eggs (2-7 mm CL) in very high numbers (*e. g.* 1000-7000), and whose hatchlings are relatively small and undergo a planktonic phase as paralarvae before settling out to the benthos (*i. e.* indirect development). The remaining five *Octopus* species are limited in their distribution to southeastern Australia. They produce large eggs (> 8 mm CL) in fewer numbers (*e. g.* 50-300), and the resulting hatchlings are relatively large and immediately adopt a benthic existence (*i. e.* direct development). Other mollusks that have shared distributions in Australia and New Zealand, and possess planktonic juvenile stages, have been reported by Wilson and Allen (1987).

Only the two *Octopus* species with planktonic juvenile stages occur in both Australia and New Zealand, suggesting that the distributional pattern in the region is the result of recent or on-going migration across the Tasman Sea, and not the result of previous land-mass connections between Australia and New Zealand. If the latter was the case, some of the species with non-planktonic stages might have been expected to occur in New Zealand as well as Australia.

Octopus species with benthic young do not have an alternate means for dispersal of individuals, and must rely on the relatively limited migration of adults. In other species with planktonic young, there is the potential for long-distance dispersal of juveniles across oceanic barriers. The Tasman Sea, with its submarine Tasman Trough at least 4000 m deep (Keast, 1959), appears to present a barrier to migration from southeastern Australia of *Octopus* species that have benthic juveniles and adults. However, species with benthic adults but planktonic paralarvae might have the potential for trans-Tasman migration. The distributional patterns of some *Octopus* species in southeastern Australia and New Zealand can thus be explained.

Pielou (1979) summarized requirements for successful transoceanic dispersal, noting the dependence on abundant offspring produced by the shore-dwelling adult, the probability of the juvenile being carried from coastal waters into open ocean circulation, the chances of the juvenile surviving the duration of the journey, and on chances of shifting back from oceanic to coastal waters after reach-

ing the other shore. These factors will be examined with respect to predictions for trans-Tasman dispersal of planktonic juvenile *Octopus*.

Octopus maorum and *O. warringa* are species whose benthic adults live in inshore waters, and whose mature females are highly fecund, producing very large numbers of small or medium sized eggs, presumably to maximize chances of at least some planktonic juveniles surviving to adulthood. A mechanism for dispersal could involve hatched planktonic young being transported via the inshore coastal currents of southeastern Australia, into larger scale offshore movements of the Southern Ocean and Tasman Sea. The Tasman Currents, derived in part from the East Australian Current and also indirectly from West Wind Drift currents, appear to be the major agents for Tasman transportation. Movement of planktonic individuals in the Tasman Sea could be facilitated by strong currents and eddies. Prevailing easterly currents across the Tasman suggest that the movement and dispersal of individuals is unidirectional from Australia to New Zealand; there is no evidence for marked movement in the opposite direction. Individuals trapped in offshore Tasman Sea eddies can experience east to west transport over reduced distances for short periods, but there is no evidence that this movement would be significant enough to transport paralarvae back to shore.

At present, the duration that juveniles of *Octopus maorum* and *O. warringa* remain in the plankton before settling out to benthic existence is unknown. An indication of planktonic life is given by studies on other representatives of the genus: Yamashita (1974) calculated that juveniles of *O. dofleini* (Wülker, 1910) remain in the plankton for 2-3 months; Villanueva (1995) and Rees and Lumby (1954) estimated that juveniles of *O. vulgaris* Cuvier, 1797, remain planktonic for around two or three months respectively. Based on these figures, juveniles of *O. maorum* and *O. warringa* could have the potential to survive as plankton in oceanic currents for three months.

If juveniles of *Octopus maorum* and *O. warringa* are to reach New Zealand from Australia, the period that the juveniles remain planktonic must be correlated with the velocity of currents flowing from Australia to New Zealand. This could determine whether the young are able to be transported across the Tasman to the New Zealand coast in sufficient time, before their transition from plankton to benthos, or whether they perish before ever reaching the coast. Rates of current flow for the East Australian Current are estimated at 2 m/sec by Hamon *et al.* (1975), and for some Tasman Sea eddies at 1.3 m/sec by Cresswell (1983). Such movements for passively drifting planktonic material are deemed maximal, and will be reduced when animals are caught in slower flowing currents or entrapped in eddies. Distances from the coast of Australia to New Zealand range from approximately 1500 to 2200 km. By calculation, the fastest period in which an organism could be transported in plankton from Australia to New Zealand, under optimal conditions, would be about nine days. Because of variable current patterns and rates of flow, it is highly improbable that transportation occurs so rapidly. Nevertheless, given that *O. maorum* and *O. warringa* individuals might survive in the plankton for three months, it seems possible that juveniles could survive suspended in the plankton long enough to reach New Zealand from Australia. Hence, despite the isolation of adult populations of the two species in Australia and New Zealand, genetic exchange could be possible by continuous or sporadic transport of juvenile individuals across the Tasman Sea.

Elucidation of the distribution of *Octopus maorum* and *O. warringa* must await detailed studies of plankton systematics and ecology from off southeastern Australia and New Zealand. Collections are at hand to study the distribution of octopus paralarvae across the Tasman Sea, but until the author has completed further studies, some of the above comments must remain speculative.

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